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Thesis submitted to Universidade Católica Portuguesa to attain the degree of PhD in Biotechnology, with specialisation in Microbiology

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by

Gisela Cristina da Cunha Mendes

under the supervision of

Cristina Luísa Miranda Silva, Associate Professor

and co-supervision of

Teresa Maria Ribeiro da Silva Brandão, Ph.D.

July 2011

"Imagination is more important than knowledge. For knowledge is limited to all we now know and understand, while imagination embraces the entire world, and all there ever will be to know and understand."

Albert Einstein

Resumo

O óxido de etileno (EO) é um agente de esterilização dominante na indústria dos dispositivos médicos, devido à sua efectividade e compatibilidade com a maioria dos materiais. Considerando o crescimento exponencial do mercado de dispositivos médicos sensíveis, complexos e sofisticados, assim como de conjuntos de procedimento customizados (que combinam uma grande diversidade de produtos e gama de polímeros) utilizados em actos médicos e cirúrgicos específicos, o EO emerge como o método de esterilização de eleição. A optimização deste processo constitui um desafio devido ao facto da competitividade do mercado global exigir custo-efectividade, flexibilidade e redução do tempo de ciclo necessário à colocação dos produtos no mercado, sem prejuízo da segurança e assegurando o cumprimento dos requisitos reguladores.

A esterilização por EO é um processo multi-paramétrico complexo, que exige uma fase final de arejamento dos materiais. A eficácia destes processos é influenciada por diversas variáveis (*e.g.* temperatura, humidade, concentração do agente, material alvo), pelo que a sua optimização exige o conhecimento da influência das condições impostas e das suas relações com os produtos.

A modelização matemática do processo de esterilização e arejamento permite a definição das condições óptimas para morte microbiana e desgaseificação, respectivamente. Tal permite a redução dos tempos de ciclo e/ou concentração de EO, assim como a comparação de diferentes processos de esterilização. Deste modo é possível contribuir para o desenvolvimento de processos com eficiência e flexibilidade acrescidas e a libertação paramétrica da esterilização surge assim cientificamente apoiada.

Nesta dissertação foi estudada a influência das condições de processo na esterilização de dispositivos médicos por EO. Este é um processo implementado na empresa Bastos Viegas, S.A., desde 2005. Foram estudados os efeitos e interacções da temperatura, concentração de óxido de etileno e humidade relativa na inactivação do *Bacillus subtilis, var. niger* (ATCC 9372), o microrganismo de referência usado no controlo do processo. As experiências foram realizadas em câmaras de esterilização, com uma carga de campos cirúrgicos. Aplicou-se um planeamento experimental factorial 2^3 para avaliação dos efeitos das três variáveis (de acordo com limites comuns de condições operacionais) na letalidade da esterilização por EO.

A inactivação do *B. subtilis* apresentou um comportamento sigmoidal e um modelo baseado em Gompertz foi ajustado com sucesso aos dados experimentais. Características importantes das curvas, tais como atraso inicial e taxa de inactivação foram considerados parâmetros do modelo. A

temperatura e a concentração de óxido de etileno foram as variáveis que afectaram significativamente esses parâmetros, pelo que experiências adicionais foram realizadas de forma a incluir o efeito dessas variáveis do processo. Foi desenvolvido um modelo matemático para previsão da morte de *B. subtilis* expresso em função da temperatura e concentração de EO.

Uma vez que a letalidade demonstrou estar directamente relacionada com a concentração de agente esterilizante, compreender a efectividade da esterilização por EO suscita obter a permeabilidade dos materiais ao gás, assim como o conhecimento sobre a dinâmica deste processo. A análise em tempo real da concentração de EO no espaço-livre do esterilizador tem-se tornado prática comum e o desafio neste momento é prever o seu valor no interior da carga. Foi desenhado, concebido e desenvolvido um equipamento para determinar a difusividade e a solubilidade de EO, e a permeabilidade de diversos materiais ao gás, pela metodologia de tempo de atraso. A operação deste equipamento é baseada no princípio de medição de uma mudança transiente de pressão em condições de volume e temperatura constantes (abordagem pressão-variável) e permite a determinação do tempo de atraso e do fluxo de EO em estado estacionário através de diversos materiais. As experiências foram executadas com um material de campo cirúrgico, a uma temperatura típica de esterilização, *i.e.* 48 $^{\circ}$C, e a uma pressão de 3,2x10^{5} Pa. As propriedades de transferência do EO no campo cirúrgico foram usadas para modelizar a sua cinética numa carga industrial do mesmo material. A segunda lei de Fick demonstrou ser adequada na descrição do processo de transferência, o que foi validado pela utilização de dosímetros que integraram a concentração de EO durante o tempo de exposição.

A etapa de arejamento é importante para reduzir os resíduos de EO a um nível seguro e neste contexto, prever a desorção do EO é uma importante ferramenta para a optimização global da esterilização por EO. A cinética de desorção de EO de diferentes materiais esterilizados foi avaliada numa gama de temperaturas de arejamento entre 1,5 e 59,0 °C. Os dados experimentais seguiram um processo de difusão Fickiano e as difusividades foram estimadas para dois materiais médicos têxteis e dois poliméricos.

Os resultados apresentados nesta dissertação contribuem para uma melhor compreensão da dinâmica do processo de esterilização por EO e por conseguinte, para uma optimização e controlo eficiente deste processo.

Palavras-chave: Dispositivos médicos, esterilização, óxido de etileno, esporos de *Bacillus subtilis,* microbiologia preditiva, célula de permeação, difusão de óxido de etileno, processo de arejamento, polímeros, materiais têxteis.

Abstract

Ethylene oxide (EO) is a dominant sterilisation agent in medical device industry, due to its effectiveness and compatibility with most materials. Considering the exponential market growth of sensitive, complex and sophisticated medical devices, as well as custom procedure packs (that combine a large diversity of products and range of polymers) for use in specific medical and surgical procedures, EO emerges as the sterilisation method of choice. The process optimisation is a challenge, due to the fact that the global competition market requires cost effectiveness, flexibility and inherent reduction of turnaround time required to get the products to market, without compromising safety and compliance with regulatory requirements.

Ethylene oxide sterilisation is a complex multi-parameter process that requires an aeration final step of the materials. The effectiveness of these processes is influenced by several variables (*e.g.* temperature, humidity, concentration of the agent, target material), so their optimisation requires knowledge of the influence of the conditions imposed and of their relations with the products. Mathematical modelling of the sterilisation and aeration processes allows the definition of optimal microbial inactivation and outgassing conditions, respectively. This allows cycle times and/or EO concentration reduction, as well as the comparison of different sterilisation processes. In this way it is possible to contribute to the development of processes with added efficiency and flexibility and, therefore, the parametric release of the sterilization arises scientifically supported.

In this dissertation the influence of process conditions on EO sterilization of medical devices was studied. This is an implemented process in Bastos Viegas, S.A. company, since 2005. The main effects and interactions of temperature, ethylene oxide concentration and relative humidity on the inactivation of the *Bacillus subtilis, var. niger* (ATCC 9372), the reference microorganism used in the control of the process, were assessed. The experiments were carried out in sterilisation chambers, with a load of surgical drapes. A 2^3 full factorial experimental design was applied for evaluation of the effects of the three variables (under the common limits of operational conditions) on microbial lethality by EO sterilisation.

The *B. subtilis* inactivation presented a sigmoidal behaviour and a Gompertz based model was successfully applied in experimental data fitting. Important characteristics of the curves, as initial shoulder and inactivation rate were considered model parameters. Temperature and ethylene oxide concentration were the variables that significantly affected those parameters, so additional

experiments were carried out to include the effects of those process variables. A mathematical model for *B. subtilis* death prediction was developed, expressed in terms of temperature and EO concentration.

Since the lethality was found to be directly related with the sterilant agent concentration, understanding EO sterilisation effectiveness requires attaining gas permeability of materials, as well as the knowledge of the process dynamics. The on-line analysis of EO concentration in the steriliser head-space is becoming common practice and the challenge now is to predict its value within the load. An apparatus was designed, conceived and developed for determination of EO diffusivity and solubility, and gas permeability of sheet materials, by lag time methodology. The operation of this apparatus is based upon the principle of measuring a transient change in pressure at conditions of constant volume and temperature (variable-pressure approach) and allows determination of lag time and steady state flow of EO through different materials. Experiments were carried out with a surgical drape material at a standard sterilisation temperature, *i.e.* 48 °C, and at a pressure of 3.22×10^5 Pa. The transport properties of EO through surgical drape were used for modelling its kinetics through an industrial load of the same material. The Fick's second law was adequate for describing the transport process, which was validated by the use of dosimeters that integrated EO concentration through exposure time.

The aeration step is important for reducing EO residues to a safe level and in this context, prediction of EO desorption is a valuable tool for the overall optimisation of EO sterilisation. The kinetics of EO desorption, from different sterilised materials, was assessed within the range of aeration temperatures from 1.5 to 59.0 °C. The experimental data followed a Fickian diffusion process and diffusivities were estimated for two textile and two polymeric medical materials.

The results presented in this dissertation contributes to a better understanding of the full dynamics of EO sterilisation and consequently, for an optimisation and efficient control of this process.

Keywords: Medical devices, sterilisation, ethylene oxide, *Bacillus subtilis* spores, predictive microbiology, permeation apparatus, ethylene oxide diffusion, aeration process, polymers, textile materials.

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The preparation of the thesis would not be possible without the support of several persons and Institutions, to whom I am very grateful. Herein I express my acknowledgment to all the ones that supported me in achieving this important goal.

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To Dr. Clark Houghtling and Dr. Gregg Mosley to whom I thank for the opportunity to exchange information and share experiences in this field, and in particular Clark, for the moments we shared. I truly admire their commitment to science.

I hereby acknowledge Bastos Viegas for financial support through a PhD grant and for giving me the opportunity to develop my work in a very promising area.

The support and cheering of my very best and closest friends was essential to keep me in the right mood during these last working years. I feel really lucky for that. I want to address them a special "Thanks!"

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I also wish to thank my brother for always bringing happiness and extra positivity to my life. My very special thanks for being such a good friend.

Finally, it is difficult to express gratefulness to someone so special to me. His love and care were essential for giving me the strength to face this challenge. Thank you for being by my side in all moments. Thank you Pedro!

Table of Contents

Appendixes

List of Symbols

Symbols

A	Tail (chapter 2); Area (chapter 3)
a and b	Model parameters that relate shoulder and maximum inactivation rate with temperature and ethylene oxide concentration (chapter 2)
C	Ethylene oxide concentration (mg L^{-1} or mol m^{-3})
$\overline{C}$	Average ethylene oxide concentration (mg L^{-1})
C*	Ethylene oxide concentration per hour (mg L^{-1} h^{-1})
CI	Confident interval
CO_2	Carbon dioxide
^{60}Co	Cobalt-60
D	Diffusion coefficient (m^2 s^{-1})
D-value	Decimal reduction time (min)
d	rise
Ea	Activation energy (J mol^{-1})
F	Equivalent process time (min)
J	Flow, rate of diffusion (m^2 min^{-1})
k	Inactivation rate constant (min^{-1})
θ	Lag time (s or min)
l	Thickness of the material (cm)
λ	Shoulder (s or min)
MM	Molecular mass
N	Microbial load
n	Total number of process steps
$\mathscr{P}$	Permeability coefficient (mol s^{-1} m^{-1} Pa^{-1})
P	Pressure (Pa or Bar)
p	Partial pressure (Pa or Bar)
Q	Amount of gas
Q_{10}	Temperature coefficient (measure of the rate of inactivation as a consequence of increasing the temperature by 10 °C).
r	Number of negative BIs

Symbols (continued)

R	Gas constant (8.314472 m^3 Pa K^{-1} mol^{-1})
R^2	Coefficient of determination
S	Solubility coefficient (mol m^{-3} Pa^{-1})
t	Time (s or min)
T	Temperature (°C or K)
U	Equivalent (exposure) time (s or min)
V	Volume (m^3)
x	Position / Distance (cm)
z	Temperature increase required for a ten-fold reduction of D-value

Subscripts and Superscripts

0	Initial value
b	Interface
d	Downstream
i	Process step / layer
int	Interior
max	Maximum value
m	Total number of process steps
n	Model parameter
R	Reference value
ss	Steady state
u	Upstream

Abbreviations

AAMI	Association for the Advancement of Medical Instrumentation
ABS	Poly(acrylonitrile-butadiene-styrene)
ANOVA	Analysis of variance
ANSI	American National Standards Institute, Inc.
ANSI/AAMI ST	Standard developed by AAMI and approved by ANSI
ATCC	American type culture collection
B.	*Bacillus*
BI	Biological indicator
BIER	Biological indicator evaluator resistometer
CDL	Cell-derived leukaemia
CFC-12	Chlorofluorocarbon-12
CFR	Code of Federal Regulations
CFU	Colony forming unit
CI	Confidence interval
CSI	Continuous steam injection
DEC	Dynamic environmental conditioning
DNA	Deoxyribonucleic acid
Dos	Colour progression in dosimeter (cm)
EBZ	Employee breathing zone
ECH	Ethylene chlorohydrin
EG	Ethylene glycol
EN	European norm
EO	Ethylene oxide
EPA	Environmental protection agency
EPCD	External process challenge device
ERPG	Emergency response planning guideline
EUCOMED	European Association of Medical Device Manufacturers
FID	Flame-ionization detector
GC	Gas chromatography
HCFCs	Hydrochlorofluorocarbons

Abbreviations (continued)

HIMA	Health Industry Manufacturers Association
HS	Head-space
HSKP	Holcomb-Spearman-Karber procedure
IDLH	Immediately dangerous to life or health
IPCD	Internal process challenge device
IR	Infrared
ISO	International Organization for Standardisation
ISO/DIS	Draft International Standard of ISO
LSKP	Limited Spearman-Karber procedure
LSMCP	Limited Stumbo-Murphy-Cochran procedure
MD	Medical device
MDA	4,4'-Methylenedianiline
NFPA	National Fire Protection Association
NIOSH	National Institute for Occupational Safety and Health
NOHSC	National Occupational Health & Safety Commission
OSHA	Occupational and Safety Health Administration
PCD	Process challenge device
PE	Polyethylene
PEL	Permissible exposure limit
PPE	Personal protective equipment
PPQ	Physical performance qualification
PSI	Pulsed steam injection
PU	Polyurethane
RH	Relative humidity
RNA	Ribonucleic acid
rpm	Rotations per minute
SAL	Sterility assurance level
SHW	Standardised half width
SLR	Spore log reduction
SMCP	Stumbo-Murphy-Cochran procedure

Abbreviations (continued)

SPME	Solid phase micro-extraction
STEL	Short term exposure limit
TIR	Technical information report
TWA	Time weighted average
UHMWPE	Ultra-high-molecular-weight-polyethylene
UK	United Kingdom
USA	United States of America
USP	United States Pharmacopeia

General Introduction

Project rationale and objectives

Ethylene oxide (EO) is the leading technology in sterilisation of medical devices (MDs) and consequently, studies in the field of process optimisation are of main importance. As a response to market demand to optimise process conditions, while simultaneously reducing the overall sterilisation process time and cost and bringing flexibility to the process, without compromising the delivery of sterile and safe products to the market, a research effort was given to this topic.

There is a considerable number of works exploiting relevant influencing factors (such as temperature, ethylene oxide concentration and humidity) on EO sterilisation. However, the mathematical modelling of the microbial lethality related to EO sterilisation, aiming at a complete description of the inactivation pattern, is scarce. Moreover, the assessment of influencing variables on microbial inactivation kinetics is undoubtedly lacking.

Studies providing an overview of EO transport properties are also limited. As EO concentration is one of the requisite process parameters for estimating the lethality of the cycle, and due the complexity of controlling EO concentration within the load, modelling of EO permeation is a key issue. The prediction of the dynamic distribution of EO concentration within the load being sterilised, would allow the estimation of theoretical sterilising EO concentrations and this is useful for understanding both sterilisation and aeration processes.

In addition, and considering the overall optimisation of EO sterilisation, the prediction of ethylene oxide desorption is important for assuring a proper aeration, contributing to an efficient removal of residual ethylene oxide content.

This works intends to contribute for attaining a better understanding of the process, by progress in the field of mathematical models to integrate lethality and EO diffusion. The parametric release procedure will certainly also be much more scientifically supported.

The main objective of this dissertation is the optimisation of process variables involved in EO sterilisation of medical devices, by mathematical modelling of *Bacillus subtilis* inactivation, allowing a characterization of microbial kinetics. This will contribute to a complete assessment of influencing factors (e.g. temperature, ethylene oxide concentration and humidity) with the potential of designing more efficient and controlled processes. In addition, understanding EO diffusivities through different medical devices for future development of mathematical models to integrate EO diffusion, is also an objective.

More specific objectives of this dissertation include:

(i) Generation of experimental data of microbial kinetic behaviour throughout the ethylene oxide sterilisation process;

(ii) Quantification of microbial inactivation kinetics by applying predictive microbiology models;

(iii) Influence of the process variables and combined effects in kinetics parameters and lethality modelling;

(iv) Design and conception of an apparatus for measuring ethylene oxide transport properties through different materials of the medical devices;

(v) Modelling of EO gas diffusion/permeation through the load;

(vi) Estimation of EO diffusivities/desorptions through different materials in a range of aeration temperatures.

Outline of dissertation structure

This dissertation is divided into six chapters, including an introduction (that intends to be a review of ethylene oxide as sterilising agent of medical devices), conclusions and suggestions for further work. The remaining chapters are organised into two major parts, schematically presented in Figure I.

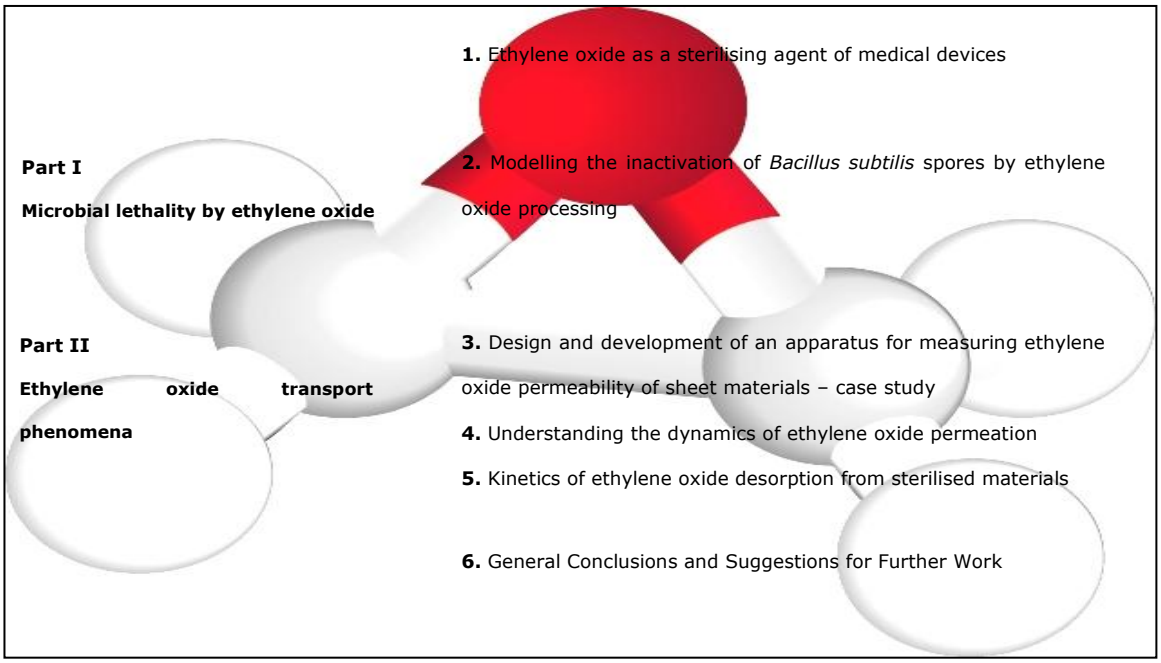


Figure I. Schematic structure of the dissertation

Part I, chapter 2, addresses a predictive microbiology work on ethylene oxide sterilisation. The experimental conditions for the assessment of the kinetics of *B. subtilis* inactivation are detailed and the process variables effects in kinetic parameters were achieved.

Part II deals with modelling of EO permeation behaviour. In chapter 3, a description of a conceived apparatus for experimental determination of ethylene oxide diffusivity through

different materials of the medical devices is described. This data will be important for attaining a global kinetic approach of gas permeation through EO sterilisation, described in chapter 4. The case study elected for validation of the model is a surgical drape load. In chapter 5, kinetics of EO desorption from different materials in a range of aeration temperatures were evaluated.

Scientific outputs of dissertation

Part of the work reported in this dissertation has already been subjected to international peer reviewing via publication in international journals, as indicated in the list below. Furthermore, the research work has been presented to the scientific community in international conferences.

LIST OF PUBLICATIONS

Book chapters

1. Mendes, G.C., Brandão, T.R.S. and Silva, C.L.M. (2011)"Ethylene Oxide Sterilization". In. Sterilization of biomaterials and medical devices, S. Lerouge and A. Simmons (Ed.), Woodhead Publishing Limited, Abington Hall, Abington, Cambridge, UK, *Invited publication*.

Peer-reviewed articles

1. Mendes, G.C., Brandão, T.R.S. and Silva, C.L.M. (2011) "Modelling the inactivation of *Bacillus subtilis* spores by ethylene oxide processing", *Journal of Industrial Microbiology and Biotechnology*, 38(9): 1535-1543.

2. Mendes, G.C., Brandão, T.R.S. and Silva, C.L.M. (2008) "Ethylene oxide potential toxicity", *Expert Review of Medical Devices*, 5(3): 323-328, *Invited paper*.

3. Mendes, G.C., Brandão, T.R.S. and Silva, C.L.M. (2007) "Ethylene oxide sterilization of medical devices: a review", *American Journal of Infection Control*, 35(9): 574-581.

Non-reviewed articles

4. Mendes, G.C., Brandão, T.R.S. and Silva, C.L.M. (2009) "Modelling the inactivation of *Bacillus subtilis* by ethylene oxide processing". *Proceedings of the symposium: "10th World Congress of Sterilization"*, Crete, Greece, 7 to 10 October.

Submitted articles

5. Mendes, G.C., Brandão, T.R.S. and Silva, C.L.M. (2011) "Kinetics of ethylene oxide desorption from sterilized materials", Journal of Pharmaceutical Sciences, *Submitted.*

6. Mendes, G.C., Brandão, T.R.S. and Silva, C.L.M. (2011) "Design and development of an apparatus for measuring ethylene oxide permeability of sheet materials", *Journal of Membrane Science, Submitted.*

Communications

Oral communications

1. Mendes, G.C., Brandão, T.R.S. and Silva, C.L.M. (2009) "Modelling the inactivation of *Bacillus subtilis* spores by ethylene oxide processing", 10th World Congress of Sterilization, Crete, Greece, 7 to 10 October.

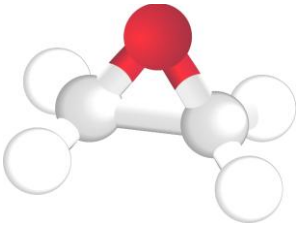
Posters communications

1. Mendes, G.C., Brandão, T.R.S. and Silva, C.L.M. (2009) "Study of the influence of ethylene oxide sterilization variables on *Bacillus subtilis* inactivation", III International Conference on Environmental, Industrial and Applied Microbiology, BioMicroWorld2009, Lisbon, Portugal, 2 to 4 December.

2. Mendes, G.C., Brandão, T.R.S. and Silva, C.L.M. (2006) "Ethylene oxide sterilization of medical devices", 20th European Conference on Biomaterials, ESB2006, Nantes, France, 27 September to 1 October.

Chapter 1

Ethylene oxide as sterilising agent of medical devices



This chapter provides a basis for contextualizing the ethylene oxide as a dominant agent in medical devices sterilisation. The advantages and disadvantages as well as its recommended uses are explored. The variables involved and their relevance to process optimisation are described, the types of processing cycles are detailed and emphasis is given to the design and validation of the sterilisation process.

1. PREAMBLE

This chapter provides a comprehensive review, divided in four parts, of the sterilisation process of medical devices by ethylene oxide.

The first part presents an overview of the mechanism of the EO activity and inherent toxicity of the sterilising agent. Regulated limits for occupational exposure, as well as for patients using EO sterilised MDs are also discussed.

The second part summarizes the advantages of this sterilisation technology over the other two industrial sterilisation technologies most widely used (irradiation and moist heat). The objective is to explain why EO, despite many predictions about its demise as a sterilisation alternative, is still a dominant mode of sterilisation and continues to be used for high volumes of MDs.

The third part outlines the process design and process validation procedures, including the microbiological validation, which is the most challenging one.

Finally, the last part addresses the state-of-the-art on optimisation of EO sterilisation conditions. Topics, such as parametric release, lethality and modelling of EO diffusion, are discussed.

1.1. INTRODUCTION

Sterilisation processes in medical field have become increasingly complex due to the need to prevent patient exposure to infections caused by contaminated instruments and/or devices. Failures in adequate sterilisation of medical devices result in significant institutional costs related to patient nosocomial infections and mortality *versus* mortality concerns (Anonymous, 1996; Rutala et al., 1999).

The appropriate sterility assurance level (SAL), which is the probability of viable microorganisms presence on a sterilised medical device, is regulated by national authorities and may vary from country to country. According to EN 556-1:2001, a SAL of 10^{-6} is a requirement for terminal sterilisation of MDs (EN 556-1, 2001; ANSI/AAMI/ ST67, 2003). However, ANSI/AAMI ST 67: 2003 refers that a SAL of 10^{-3} can be used for certain MDs, depending on their intended use or on their inability to withstand a terminal sterilisation process that provides a SAL of 10^{-6}.

The more widely used industrial MDs sterilisation technologies are moist heat, ethylene oxide, gamma and electron beam irradiation. Until the introduction of EO sterilisation in the latest 1950s, dry heat and moist heat sterilisation were the only methods available to routinely sterilise medical devices. Gamma and electron beam irradiation are much more recent technologies. Other methods, such as low-temperature hydrogen peroxide gas plasma, low-temperature peracetic acid gas plasma, vapour-phase hydrogen peroxide, ozone, chlorine dioxide, and high-intensity visible light are not entirely assessed (Anonymous, 1996; Feldman and Hui, 1997; Rutala et al., 1998; Fairand et al., 2003).

Ethylene oxide has emerged as the sterilisation method of choice for MDs, due to its undeniable advantages when compared with other technologies. Ethylene oxide is an exceptional sterilising agent, due to its effective bactericidal, sporicidal and virucidal

activity. However, difficulties had to be overcome, mostly related with potential hazards of EO to patients, staff and environmental, as well as risks associated with handling a flammable gas (Barker, 1995; Anonymous, 1996; Rutala and Weber, 1999; Hucker and Axel, 2001; Strain and Young, 2004). In fact, during the latter half of the 1980s and the early 1990s, the future of EO sterilisation used in the health care industry was strongly questioned, basically due to the disadvantages above referred. In 1989, the Eucomed EO sterilisation conference was organized to demonstrate that EO sterilisation remained a safe and effective technology. Ethylene oxide has allowed and contributed significantly to the advancement and evolution of delicate, complex and sophisticated MDs, that otherwise would not be available. For sensitive materials, EO is the only acceptable sterilisation method (Barker, 1995; Anonymous, 1996). Ethylene oxide sterilisation became even more important since the single-use MD market has grown. With the purpose of cost-saving in health management, there has been a transition to develop MDs in customized packs, for use in specific medical and surgical procedures. According to Barker (1995) and Strain and Young (2004), the diversity of developed products, designs, types of materials and packaging configurations, resulted in an exponential growth of EO sterilisation. This made EO the agent most widely used in MDs sterilisation processes, with a continuous growth tendency.

Nowadays, EO sterilisation is described as the most cost-effective low temperature sterilisation process available, with a recognized history of reliability (Barker, 1995; Hucker and Axel, 2001; Sordellini et al., 2001; Mosley et al., 2002; Strain and Young, 2004).

The aim of this chapter is to gather information on EO sterilisation, and to present critical points of view about what has been done and what is required, in order to provide the tools for advancement and optimisation of EO sterilisation of MDs.

There are several guidelines, standards and other documents, which provide a consensual understanding on implementation of harmonized methods related to EO sterilisation. These guideline documents have been classified into three main issues (see Table 1.1) that are related with the sterilising agent, equipment considerations and the process itself. It should also be mentioned that some of these documents are not specific, and include guidelines for all the referred issues.

Table 1.1 Ethylene oxide sterilisation guideline standards and related documents

Issue		Summary / Contents	Reference
	Agent	General requirements for the characterization of a sterilizing agent.	ISO 14937 (2009)
	Equipment	Sterilizers for medical purposes – Ethylene oxide sterilizers – Requirements and test methods.	EN 1422 (2007)+A1 (2009)
		EO sterilization equipment.	AAMI TIR 15 (2009)
Process	Process design	General sterilization requirements.	ISO 14937 (2009)
		Requirements for a terminally sterilized MD, labelled "STERILE" (guidance for selection of an appropriate sterility assurance level and acceptance criteria for maximum contamination rates).	ANSI/AAMI ST 67 (2003) ANSI/AAMI ST 67 (2003)/R (2008)
		Compatibility of materials subject to sterilization.	EN 556-1 (2001)
		Process considerations and pertinent calculations.	AAMI TIR 15 (2009)
		Microbiological aspects of the development of an ethylene oxide sterilization process.	AAMI TIR 16 (2009)
		Guidance for the selection, use and interpretation of results of biologic indicators, when used in the development of sterilization processes.	ISO 14161 (2009)
		Approach for the implementation and development of parametric release.	AAMI TIR 20 (2001)
		Guidance for the adoption of a new or modified product into an existing validated sterilization process, and for the determination of equivalency of a sterilization process as conducted in different equipments.	AAMI TIR 28 (2009)

Table 1.1 (continued) Ethylene oxide sterilisation guideline standards and related documents

Process	General requirements	General process requirements and guidance.	ISO 14937 (2009) ISO 11135-1 (2007) ISO /TS 11135-2 (2008) ISO/TS 11135-2/Cor 1 (2009) EN 550 (1994)
	Chemical indicators	General performance requirements and acceptance criteria for chemical indicators.	ISO 11140-1 (2005) ANSI/AAMI/ISO 11140-1 (2005)/R (2010)
		Guidance for the selection, use and interpretation of chemical indicators that are intended to monitor sterilization processes.	ANSI/AAMI/ISO 15882 (2008) ANSI/AAMI ST 60 (1996)
	Microbiological aspects	Microbiological aspects of the validation of an ethylene oxide sterilization process.	AAMI TIR 16 (2009)
		Guidance on selecting a microbial challenge and inoculation sites for sterilization validation of medical devices.	AAMI TIR 39 (2009)
	Biological indicators	Guidance for the selection, use and interpretation of results from BIs.	ISO 14161 (2009)
		General requirements of BI systems to be used in the validation and routine monitoring of sterilization processes.	ISO 11138-1 (2006)
		Specific requirements for test organisms, BI systems and test methods intended for use in assessing the performance of sterilizers and sterilization processes employing EO gas as the sterilizing agent.	ISO 11138-2 (2006) ANSI/AAMI/ISO 11138-2 (2006)/R (2010) ANSI/AAMI ST 21 (1994)
		Guidance for the selection, use and interpretation of results of biologic indicators when used to validate and monitor sterilization process.	ISO 14161 (2009) ANSI/AAMI ST 59 (1999)
	Bioburden	Requirements and guidance for the enumeration and characterization of the population of viable microorganisms on or in medical device, component, raw material or package.	ISO 11737-1 (2006) ISO 11737-1/Cor 1 (2007)
	Sterility tests	General criteria for tests of sterility on medical devices that have been exposed to a treatment with the sterilizing agent that is a fraction of the specified sterilization process. These tests are intended to be performed when validating sterilization process.	ISO 11737-2 (2009)
	Parametric release	Approach for the validation of parametric release.	AAMI TIR 20 (2001)

Table 1.1 (continued) Ethylene oxide sterilisation guideline standards and related documents

Process		Allowable limits for residual EO and ECH in individual EO-sterilized medical devices, procedures for the measurement of EO and ECH, and methods for determining compliance so that devices may be released.	ISO 10993-7 (2008)
	Residues	Guidance to augment ANSI/AAMI/ISO 10993-7. Assists in understanding the steps necessary to evaluate an ethylene oxide-sterilized device according to the ANSI/AAMI/ISO 10993-7. Also provides limited guidance for the application of other parts of the ANSI/AAMI/ISO 10993 series of standards to the biologic evaluation of ethylene oxide-sterilized MDs.	AAMI TIR 19 (1998) AAMI TIR 19/A1 (1999)
	Contract sterilisation	Guidance for both medical device manufacturers that use contract sterilization facilities and contract sterilization operations.	AAMI TIR 14 (2009)
	Safety	Requirements for work place safety.	29 CFR, part 1910.1047 (1997) NOHSC 3016 (1992)
		Requirements for personal protective equipment.	29 CFR Part 1910.132 29 CFR Part 1910.133
		Ethylene oxide sterilization safety in the health care facilities.	AAMI ST 41 (2008)
		Operator safety.	EN 61010-1/A2 (1995) IEC 1010-2-042 (1997)
		Prevention of worker injuries and deaths from explosions in industrial ethylene oxide sterilization facilities.	NIOSH Safety Alert (2000)

1.2. ETHYLENE OXIDE PROPERTIES, STERILISATION MECHANISM AND TOXICITY

1.2.1. Ethylene oxide properties

As reported by the French Academy of Sciences, ethylene oxide, 1,2-epoxy-ethane, was firstly synthesized in 1859 by Wurtz (Bommer and Ritz, 1987). However, it was only developed as sterilant in the 40's by the military and in the 50's the McDonald process was patented for use in MDs sterilisation (Fairand et al., 2003).

Table 1.2 included some of the EO most important properties, which will help further understanding of the contents of this dissertation.

Table 1.2 Ethylene oxide properties

Property		Behaviour	Reference
Physicochemical			
Boiling point	10.7 °C	Gas at room temperature and atmospheric pressure Highly volatile and reactive, flammable and potentially explosive gas at ordinary room temperature and pressure	Bommer and Ritz (1987) Rutala and Weber (1999) Tsai et al. (2004) 29 CFR Part 1910.1047 (1997) The Merck Index (1983)
Explosive limits	3-100 %		
Flash point	-55 °C		
Auto ignition temperature	429 °C		
Exergonic combustion reaction	313 kcal mol^{-1}		
Solubility	Miscible with water and organic solvents	Allows aqueous extraction of residues	The Merck Index (1983)
Sensorial			
Colour	Colourless gas	Despite the ether-like odour, it is not a reliable sensorial warning of EO's hazardous due to its high odour threshold	Rutala and Weber (1999)
Odour	Ether-like		
Microbiological activity			
Range of activity	Bactericidal, sporicidal and virucidal	Exceptional sterilizing agent	Rutala and Weber (1999)
Health effects			
Acute effects	Respiratory and eye irritation, headache, nausea, vomiting, and diarrhoea	Requires highly trained operations, efficient management and good plant infrastructures in order to follow safe work practices	Yahata et al. (2001) Tsai and Wu (2003) Tsai et al. (2004) NIOSH Current Intelligence Bulletin 35 (1981)
Chronic effects	Altered behaviour, anaemia, secondary respiratory infections, skin sensitisation, miscarriages, and reproductive problems		
	Carcinogen		

1.2.2. Ethylene oxide sterilisation mechanism

The EO high reactivity, as expressed by the high energy of its exergonic combustion reaction (see Table 1.2), in combination with its high diffusivity, is of major importance for the inactivation of microorganisms (Phillips and Miller, 1973; Bommer and Ritz, 1987). Ethylene oxide is a direct alkylating agent that does not require metabolic activation. Its microbiological inactivation properties are considered to be the result of its powerful alkylation reaction with cellular constituents of organisms, such as nucleic acid and functional proteins, including enzymes, which leads to consequent denaturation.

The addition of alkyl groups to proteins, DNA and RNA in microorganisms by binding to the sulfhydryl and hydroxyl, amino and carboxyl groups, prevents normal cellular metabolism and ability to reproduce. This affects microbes viability (Poothulil et al., 1975; Grammer et al., 1985; Bommer and Ritz, 1987; D'Ambrosio et al., 1997; Rutala and Weber, 1999; Swenberg et al., 2000). These chemical moieties are not present in most of the MDs composition; therefore, exposure to EO does not cause them similar structural changes (Fairand et al., 2003).

1.2.3. Ethylene oxide toxicity

Taking into account the previously described EO sterilisation mechanism, it is easy to understand EO toxicity as a chemical agent, and potential related problems with employee, patient, and environmental safety.

Ethylene oxide toxicity in workplace

The ability of a chemical to serve as an alkylating agent, and to cause mutations in a variety of biological test systems, is widely accepted as an indicator of the carcinogenic potential of the chemical. Both alkylation and mutagenicity potential have been demonstrated for EO. The National Institute for Occupational Safety and Health (NIOSH) recommends EO to be regarded in the workplace as a potential occupational carcinogen (NIOSH Current Intelligence Bulletin 35, 1981; Angerer et al., 1998).

However, in nowadays, EO can be used safely with minimal personal risk of chemical hazardous exposure, by following recommended practices and meeting current Occupational Safety and Health Administration (OSHA) EO regulations (see Table 1.1; Strain and Young, 2004). The 29 CFR Part 1910.1047 (1997), besides establishing the permissible occupational exposure limits, also gives orientations for compliance with the established limits and how to monitor exposure, classify and manage regulated areas, specifying the personal protective equipment, including respiratory protection that must be used. Furthermore, NIOSH established EO concentrations dangerous to life or health (see Table 1.3).

Table 1.3 Exposure limits and EO concentration dangerous to life (or health), according to OSHA's and NIOSH's guidelines, respectively

Definition		Exposure limit	Reference
Concentration of airborne EO calculated as an eight-hour time-weighted average	PEL (permissible exposure limit)	1 ppm	29 CFR Part 1910.1047 (1997)
	Action level	0.5 ppm	
Concentration of airborne EO calculated as 15 minutes time weighted average	STEL (short-term exposure limit)	5 ppm	29 CFR Part 1910.1047 (1997)
Concentration of EO immediately dangerous to life or health	IDLH (immediately dangerous to life or health)	800 ppm	NOHSC 3016 (1992)
Maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to 1 hour without experiencing or developing irreversible or other serious health effects, or symptoms which could impair an individual's ability to take protective action	ERPG-2 (Emergency response planning guideline)	50 ppm	NIOSH Current Intelligence Bulletin 35 (1981)

Despite the discussions about EO potential risk, this sterilant is being used with greater frequency. This is due to investments on equipment that have dramatically improved the efficiency of the process and worker exposure. The process equipment of modern plants generally consists on tightly closed, highly automated and controlled systems (NOHSC 3016, 1992; Anonymous, 1996; Rutala and Weber, 1999; Hucker and Axel, 2001).

Ethylene oxide toxicity in sterilised MDs – control of residues

In addition to the problems associated with EO toxicity in workplace, it is also important to take into consideration the EO and its secondary product residuals and toxicity on sterilised MDs. Ethylene oxide and some of its derivates, such as ethylene chlorohydrin (ECH), which appears when chloride ions are present, and ethylene glycol (EG), formed by EO reaction with water, are toxic residues. Ethylene oxide itself can also be polymerised to form polyethylene glycol or polyethylene oxide and epichlorohydrin, but at least the first two secondary products are non-toxic polymers (Poothulil et al., 1975; Furuhashi and Miyamae, 1982; Buben et al., 1999).

The trace amounts of EO and its secondary products remaining on MDs depend on the type of material sterilised, including shape, thickness, density and manufacture of the basic raw material, as well as sterilisation process and aeration method used. On the one hand, thicker materials hold more EO and take longer to aerate, because the diffusivity path is longer; but on the other hand, harder materials retain lower EO concentrations, but also take longer time to aerate. Furthermore, it is known, for example, that polyvinyl chloride, polystyrene and rubber retain more ethylene oxide products than polyethylene, polyurethane, silicone, ABS (Acrylic Butyl Styrene) and polycarbonate. These last materials retain more EO than nylon, paper or cotton. Metal and glass are two materials that retain very low quantities of EO products (Poothulil et al., 1975; Aeschlimann, 1986; Buben et al., 1999; Lucaset al., 2003).

Taking into consideration the diversity of MDs sterilised with EO, and the potential undesirable effect on patient health, residue control is required. The accurate determination of residues is also critical for the development of reliable risk assessment data used by toxicologists, epidemiologists, MD manufacturers, and regulatory bodies (Centola et al., 2001).

The series of standards governing the biological testing of MDs include the ISO 10993-7 (Biological Evaluation of MDs - Ethylene Oxide Sterilisation Residuals). It specifies the allowable limits of EO and ECH by categorization of products based on examination of toxicological risk of the residue to patient, according to the length of the time the patient is likely to be exposed to the device (limited exposure - daily, prolonged exposure - monthly, permanent exposure and special situations). The standard also outlines suitable methods for the extraction of residues from products, using exhaustive extraction and simulated use procedures, details the subsequent analysis via gas liquid chromatography, and provides the procedures for determining compliance and subsequent release to the market. The objective of simulated use procedure is to quantify the "bioavailable" EO residues, which is the amount of EO that may be assimilated by the body, and the extraction is carried under conditions that represent the intended use of MD (ISO 10993-7, 2008). With exhaustive extraction (thermal extraction followed by head-space analysis and solvent extraction procedures with either head-space gas analyser or chromatography of the solvent extract) the intention is to recover the entire residual content of a device.

The Standard Working Group 63, Sterilisation residuals working group, from the Association for the Advancement of Medical Instrumentation, undertook the task of studying variables that influence the test results for the amount of residual EO and ECH in MDs, and developed a protocol for controlling the relevant factors. The data from the study suggest that small and random variations in the technique during short sample extraction times can lead to great variability in the results. Variables such as initial water temperature, oven temperature, weighting of sample, and length of extraction should be carefully controlled. Inherent variations in the material composition of similar devices are also a possible contributing factor for the variability of test results regarding the amount of residual EO and ECH in MDs (AAMI TIR No. 19, 1998; AAMI TIR 19/A1, 1999; Centola et al., 2001).

Lucas et al. (2003) evaluated the effects of various extraction conditions and the number of re-sterilisation cycles on the amount of EO residues. In addition, the total amount of EO using exhaustive extraction was compared to the amount of EO using simulated extraction. For some polymers there was no difference between total EO residues to EO bioavailable, while others had almost eight times of difference.

All these conclusions alert for the need to carefully study the residuals determination technique. There are no general rules and each specific material has its own characteristics.

Recently, contrasting with the head-space (HS) analysis of EO residues described on ISO 10993-7, a new extraction technique called solid-phase micro-extraction (SPME) was referred by Pawliszyn. SPME presents many advantages over conventional analytical methods by combining sampling, pre-concentration and direct transfer of the analyte into a standard gas chromatograph. Determination of EO, ECH and EG in the HS gas phase, as well as in the aqueous solution, by SPME device have been referred in works of Grushka and Bar-Ilan (2001), Tsai and Wu (2003) and Tsai et al. (2004).

Just as EO diffusivity has been a well studied field, so then MD design, including the selection of materials that will follow EO sterilisation, can be done in a similar logical way. Moreover, investigations about the efficacy of different aeration methods are required.

Different aeration technologies have been reported, such as pulsed vacuums post process and heat addition, steam addition and removal, as well as combinations of different gases and pressure set points and newer developments, such as microwave desorption. Despite the major interest on this issue, there are no recent published studies about efficiency comparisons for the different aeration technologies, and current studies only show that a mechanical aeration at higher temperatures allows easier desorption of the EO residues (Peacock, 1999).

A specific study about the potential toxicity of ethylene oxide is presented in *Appendix A*.

1.3. ETHYLENE OXIDE STERILISATION AND ITS SPECIFIC ADVANTAGES

The compatibility of EO with a wide range of materials and its chemical molecule penetration properties in not so aggressive environments, compared with dry heat or moist heat, made EO sterilisation the most suitable process for the majority of heat- and/or moisture sensitive medical products (Baier et al., 1982; Angerer et al., 1998).

The effectiveness of EO sterilisation, coupled with the flexibility of the process allowed by the large number of possible control variables, represent some of the advantages of this method (Bommer and Ritz, 1987; Rutala and Weber, 1999; Hucker and Axel, 2001; Mosley et al., 2002; Strain and Young, 2004). Taking into consideration the thermal or moisture sensitivity of the specific material, the parameters of the EO cycle can be adjusted in order to preserve the integrity of the device. For many medical devices (MDs), and in particular, thermolabile plastic and elastomer polymeric materials, as well as most electronic devices and biomaterials, EO is the method of choice and sometimes the only acceptable sterilisation method (Ernest, 1973; Handlos, 1980; Rogers, 2005). Considering the exponential market growth of custom procedure packs for use in specific medical and surgical procedures that combine a diversity of products and range of polymers, the probability of encountering a material that is incompatible with a specific process increases, resulting in a exponential growth of EO applications.

Moist heat and γ irradiation sterilisation frequently cause polymers degradation and changes in physical or mechanical properties, which can be detrimental for intended performance (Fray et al., 2000; Gorna and Gogolewski, 2003; Anonymous, 2006a). Many MDs are composed of heat-sensitive materials. Regarding γ irradiation, generation of free

radicals through haemolytic bond cleavage occurs because ^{60}Co sources supply 1.17 and 1.33 MeV photon energies, which correspond to 5 orders of magnitude larger than the average energy of a chemical bond (Baier et al., 1982; Goldman and Gronsky, 1996; Ries et al., 1996; Kurtz et al., 1999; Affatato et al., 2002; Costa et al., 2002).

The opinion of some sterilisation specialists on new developments in the MDs sterilisation techniques that use oxidizing agents such as hydrogen peroxide, ozone, peracetic acid, and chlorine dioxide is that limited development will occur because of inherent adverse effects on material properties (Feldman and Hui, 1997).

1.4. FROM PROCESS DESIGN TO MARKET RELEASE OF ETHYLENE OXIDE STERILISED MEDICAL DEVICES

Process design includes the planning of the physical parameter conditions (such as temperature, humidity and EO concentration) of a sterilisation process, taking into consideration the limitations imposed by the product, the sterile barrier packaging and the equipment (Sordellini et al., 1998).

The efficiency and profitability of the process, the personnel safety and equipment integrity, as well as the well-being of the end-user of EO- sterilised MDs are all directly related to the cycle design. Regarding efficiency, the influence of each of the EO sterilisation process parameters on reaction kinetics should be considered when a cycle design is being conducted. Modelling of accumulated lethality and EO diffusion kinetics are two important issues that will be further discussed in more detail.

The sterilisation process must consistently assure that all critical process parameters are delivered within the load, to a degree that assures the required sterility assurance level

(SAL), without causing any deleterious effect on product and its sterile barrier package functionality and safety (Sordellini et al., 1998; AAMI TIR 20, 2001; ISO 14161, 2009). These activities are part of the process validation, which includes physical and microbiological performance qualifications.

The release of the MDs to the market is done according to the process specifications defined during validation. There are two methodologies for market release of MDs: conventional and parametric. The conventional traditional method of release requires that the process parameters are within the validated tolerance and the biological indicators (BIs) exposed to the sterilisation process are inactivated. On the other hand, parametric release relies solely on the recording and evaluation of the process parameters, since the equipment potentialities are enough to evaluate the impact of process parameters on microbiological inactivation (AAMI TIR 20, 2001). Parametric release is still a very challenging topic and will be further discussed in more detail.

A well structured process design, its rigorous validation, and a strictly and efficient control of the sterilisation cycle parameters are the key principles for the safe and efficacious release of the MDs to the market.

1.4.1. Process design

Investigation of all process variables impact is mandatory for reaching a well structured EO sterilisation process. Furthermore, considerations about product compatibility together with optimisation of the sterilisation process should be undertaken.

The lethality of EO sterilisation depends on the following four process parameters: (i) EO concentration, (ii) exposure time, (iii) temperature and (iv) humidity (Sordellini et al., 1998; Rutala and Weber, 1999; Heider et al., 2002; Mosley et al., 2002).

The increase of EO concentration within certain limits results in extended microbial inactivation and exposure time decrease (Rutala and Weber, 1999; Mosley et al., 2002). Heider et al. (2002) found a first order kinetics behaviour across the entire concentration range, from 50 to 1200 mg L^{-1}. In the past, concentrations of EO up to 1200 mg L^{-1} were common; while today, Heider et al. (2002) and Fairand et al. (2003) reports EO concentrations of even less than 300 mg L^{-1}. A progressive decrease of EO concentration levels have been verified, which results in shorter aeration periods after sterilisation and additional environmental, health, and safety benefits.

Temperature is an extremely important parameter, affecting microbial lethality (Heider et al., 2002; Mosley et al., 2002). Table 1.4 presents published Q_{10} and related z-values. A consensus seems to have involved a Q_{10} value of 2, which means that a 10 °C change would affect lethality by a factor of 2.

Table 1.4 Effect of temperature on EO inactivation – Q_{10} and relate z-values

Reference	**Q_{10}**	**z-value** $\left(=\frac{10\,^{\circ}C}{\log Q_{10}}\right)$
Ernest (1973)	1.80	39.2 °C
Plug et al. (2001)	1.90	36.0 °C
Lui (1968); Bruch (1981)	2.19	29.4 °C
Mosley et al. (2002)	2.21	29.0 °C

Environmental humidity appears to be another critical variable; however there were always differing opinions about the required optimum relative humidity, RH (Phillips and Miller, 1973; Oxborrow et al., 1983; Rodriguez et al., 2001). Most of the recent studies indicate that within the limits of 30-90 %, RH does not influence lethality. Sterilisation efficacy decreases markedly below 30 % and above 90 %, as RH is critical for the EO diffusivity into devices and microbes (Phillips and Miller, 1973; Mosley et al., 2002). Heider et al. (2002) found a correlation between the reaction kinetics rate and the RH in the 10 to 60 % range, whereas no further changes were observed at higher levels. According to this, it is recommended to ensure a RH of more than 60 %, so that the effectiveness of the sterilisation process is not compromised. Although nowadays it is considered that the relative humidity effect is constant, if the parameter is within the limits of 30-90 %, additional studies should be further performed to assure the true veracity of this information (Mosley et al., 2002).

The mathematical model of the reaction kinetics in EO sterilisation processes is described later under the lethality modelling topic.

Besides the EO sterilisation process parameters influence process design, other variables, such as natural bioburden, device/package properties, load density and configuration in which the specific MD is included for sterilisation, should be considered. Within the device/package properties, the raw material composition, materials diffusion properties, sensitivity to both negative and positive pressure changes, maximum allowed heat and moisture, as well as chemical tolerance to EO should be analysed (Heider et al., 2002; Mosley et al., 2002).

1.4.2. Description of a typical process

The overall EO sterilisation process includes the following three stages: preconditioning, sterilisation itself and aeration. However, an EO process may include just the sterilisation phase. During preconditioning, the devices (and the microorganisms) are exposed to increased relative humidity and temperatures, which are critical parameters for the lethality of the process. During the sterilisation stage, devices are exposed to EO gas under specified conditions, with the purpose of achieving the desired product SAL. After sterilisation, products are submitted to aeration, allowing EO residuals reduction.

In order to allow a better understanding of the critical parameters impact in each part of the process, an example of a typical EO sterilisation cycle will be explained in more detail. Figure 1.1 outlines an hypothetical and typical 100 % EO process cycle.

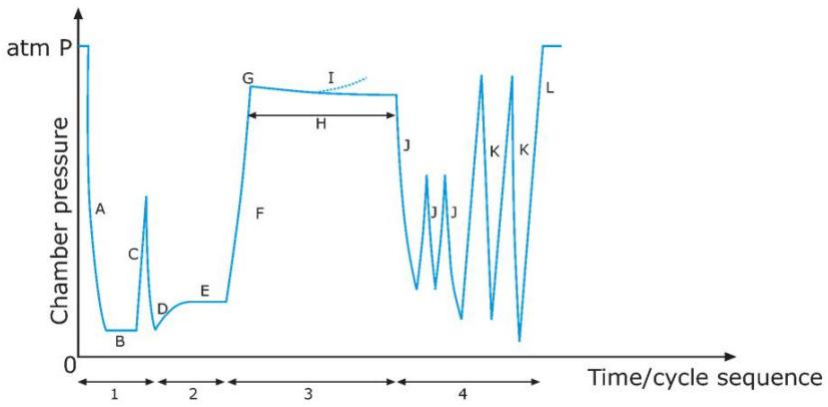


Figure 1.1 A hypothetical and typical 100 % EO process

The use of "pure" EO as sterilising agent is becoming more and more common, together with nitrogen as inert gas in order to curtail the risk of explosion. The under vacuum cycles are always the preferred choice for reducing potential hazards (EN 550, 1994; Sordellini et al., 1998; Heider et al., 2002; Fairand et al., 2003; ISO 11135-1, 2007). A typical 100 % EO process cycle (the sterilisation phase, above referred) involves: 1) initial evacuation, 2) humidity injection and humidification (conditioning) dwell, 3) EO injection and gas dwell (exposure), and 4) sterilant evacuations and nitrogen/air washes (Figure 1.1).

The purpose of the initial vacuum (A) is to remove air from the load, which on the one hand allows better penetration of humidity and EO into the interior of the load, and on the other avoids explosive environment. At this stage, product and package tolerance to deep vacuum should be taken into consideration. Moreover, if the process includes preconditioning prior to process cycle, the consequent product moisture loss should also be over viewed. After the initial vacuum, a leak test (B) is required in order to control chamber tightness. During this step deep vacuum is sustained under defined limits of time and pressure, in order to detect if an unexpected increase of pressure happens. In order to avoid very deep vacuums, a nitrogen injection (C) and subsequent vacuum allows the air remotion from the load, creating a non-explosive environment suitable for EO injection. The objective of these initial steps is to displace air as efficiently as possible and simultaneously to minimize load desiccation. A depth and fast inlet of nitrogen in the sterilising chamber minimizes product moisture loss.

During humidification, injection of moisture (D) in the form of pulsed steam is performed in order to reach the desired RH. During humidity dwell (E), to keep the chamber moisture stable even after product water absorption, steam is injected. The load dwells for a period of time, in order to allow equilibrium under the new conditions. Relative humidity determination inside a steriliser can be obtained by calculation based on

pressure rise and temperature, through dew-point determination, or by direct head-space analysis (EN 550, 1994; Sordellini et al., 1998; Fairand et al., 2003; ISO 11135-1, 2007).

Given the appropriate temperature and relative humidity uniformity, EO gas injection (F) takes place, and the degree of pressure rise determines the resulting concentration of EO in the chamber. The set point (G) can be monitored either by: calculation based on pressure change; calculation based on weight of EO injected in the chamber volume; or through direct analysis of steriliser head-space by infrared (IR) spectroscopy, gas chromatography (GC) or microwave spectroscopy (Matthews et al., 1998; Sordellini et al., 1998). An IR spectrometer allows the simultaneous monitoring of more than one analyte gas, and can be very advantageous to continuously monitor chamber steriliser water vapour and EO concentration (Zhu et al., 1993; Zhu et al., 1996; Sordellini, 1997; Zhu et al., 1999).

Gas dwell (H) provides time for EO penetration into the most difficult portions of the load, and to inactivate the microbes. Due to product/packaging absorption, EO may be added to keep the chamber EO concentration stable, known as "make-up" stage (I).

After sterilisation, EO must be removed from the chamber and from the load – sterilant evacuations. Before the steriliser is unloaded, post vacuums/nitrogen flushes (J) follow until EO concentration is brought to a safe level, i.e. below the explosion limit of 3 % (see Table 1.2). Thereafter, post vacuums are followed by air flushes (K). EO residuals absorbed and adsorbed will also lower, which makes product safer to handle and decreases the amount of time the load must be quarantined for aeration. However, the degree and number of vacuums will create negative effects on product functionality and package integrity, and these effects have to be studied in parallel with the advantages of gas washes.

Finally, air inlet (L) is required in order to bring chamber to atmospheric pressure and allow the chamber door opening (EN 550, 1994; Sordellini et al., 1998; Fairand et al., 2003; ISO 11135-1, 2007).

In case of need, a further aeration, should take place in a controlled environment. Controlled environments are required because, besides the safe working conditions that must be followed, only under strictly controlled and validated conditions it is possible to study the residues diffusion kinetics through dissipation curves that allow extrapolation the EO residual results. The validation of not only sterilisation but also the aeration process, with consequent assessment of EO residues in compliance with the requirements of ISO 10993-7, also allows a reduction in the processing time (29 CFR Part 1910.1047, 1997; ISO 10993-7, 2008).

1.4.3. Process validation

The validation of EO sterilisation processes, which includes physical and microbiological performance qualification, is described in detail in EN 550:1994 and ISO 11135-1: 2007. However, none of these norms include guidance for the selection of a sterilisation process challenge device (PCD), to be used as representative worst case matrix. Manufacture conditions, construction materials and product design, including materials geometric variability and packaging characteristics, are among the factors that need to be considered. However, the way to relate all these variables is a great challenge for the sterilisation specialist.

The physical performance qualification allows the verification of the cycle reproducibility, as well as evaluation of the cycle impact on the product, packaging functionality and safety (AAMI TIR 20, 2001).

The purpose of microbiological validation is to assess the microbiological lethality of the sterilisation process (Mosley and Houghtling, 2005; ISO 14161, 2009). The above referred International and European norms, as well as other guideline documents reported in Table 1.1, provide different approaches for carrying out microbiological performance qualification, and will be described in more detail (EN 550, 1994; ISO 11135-1, 2007).

Microbiological validation

There are three microbiological approaches for process definition, which are by decreasing order of utilization:

- Overkill method;

- Combined biological indicator (BI)/bioburden method;

- Bioburden method.

The overkill approach uses BI data to assess the microbial inactivation rate for a given process. The process definition based on "Combined biological indicator (BI)/bioburden method" defines the treatment extent required to achieve the specified SAL, from knowledge of the BIs inactivation and of the product bioburden population to be sterilised. The "Bioburden method" is a process definition based on inactivation of the microbial population in its natural state (ISO 14937, 2009).

The overkill method is applicable as long as the combination of population (10^6 microorganisms of *Bacillus subtilis var. niger*, reclassified by Fritze and Rudiger (2001) as *Bacillus atrophaeus* and resistance of the BI, expressed as D-value, exceeds that of the product bioburden. The sterilisation process definition based on this approach is often conservative, because necessary sterilisation cycle parameters are significantly higher than those required to kill product bioburden. Cycle lethality determination can be obtained from the half-cycle method, which consists of determining the minimum time of exposure at which there are no survivors from tested BIs. According to this method, at least a 6-log reduction in population of microorganisms is demonstrated for the BI organism in the half-cycle. Using the same process parameters, except exposure time,

full sterilisation cycle achieves at least a 12-log reduction by doubling the half cycle time. No D-value calculations are performed and due to its simplicity, as well to the robust SAL that is achieved, this is probably the most popular approach (EN 550, 1994; Rutala and Weber, 1999; Fritze and Rudiger, 2001; Heider et al., 2002; Mosley et al., 2002; ISO 11135-1, 2007; AAMI TIR 16, 2009; ISO 14937, 2009; ISO 14161, 2009).

The advantage of the other two microbiological approaches, combined BI/bioburden method and bioburden method, is a reduction in cycle exposure time and, consequently, the product exposure to the sterilising agent is minimized.

The combined biological indicator and bioburden method requires low population of the product bioburden and the microorganism's resistance to be known, as well as high level of confidence that the bioburden data is representative of "worst case conditions". The process definition based on the bioburden method requires extensive testing during the development phase and routine processing. It requires a validated bioburden recovery method and identification of the microorganisms that are typically found in or on the routine product, as well as more exigent environmental and manufacturing process control. Furthermore, it is necessary to carry out fractional exposure cycles on a regular basis to support the continued effectiveness of the sterilisation process. Despite the extensive work that the bioburden method requires, it may be a requirement if there is a reason to believe that the MD may be contaminated with microorganisms more resistant than the BI (AAMI TIR 20, 2001; Mosley et al., 2002; AAMI TIR 16, 2009; ISO 14937, 2009; ISO 14161, 2009).

Besides the half-cycle method, there are other two commonly used methods for estimating or calculating cycle lethality: the survivor curve method and the fraction-negative method. The survivor curve construction method calculates cycle lethality based on enumeration of survival microorganisms, and the fraction negative method uses growth/no growth data from the sterility tests.

The survivor curve construction is performed by counting microbiological survivors in terms of colony forming units, recovered after exposing the microbiological population to sub lethal sterilising cycles of graded exposures of EO, with all other parameters except time remaining constant. The survivor curve construction should include at least five points, imposing increasing exposure times to EO, and the resulting data gives the EO exposure time required to achieve a particular probability of survival of the test organism (ISO 11138-1, 2006; ISO 11135-1, 2007; ISO 14161, 2009).

The fraction-negative method is also carried out by exposing BIs to sub lethal cycles, but the analysis is done to growth/no growth data from the sterility tests (ISO 11138-1, 2006; ISO 11135-1, 2007; AAMI TIR 16, 2009).

The exposure time required to achieve a specified survival probability of the test organism is calculated from the D-value, using the limited Spearman-Karber procedure (LSKP), which is the common reference method for International Standards. However, there are other commonly used statistical methods, such as the Holcomb-Spearman-Karber procedure (HSKP), the Stumbo-Murphy-Cochran procedure (SMCP), or the limited Stumbo-Murphy-Cochran procedure (LSMCP), which can be used under particular conditions (ISO 11138-1, 2006; ISO 14161, 2009). Although an ISO meeting suggested that the SMCP was less accurate than the LSKP, and ISO recommended abandoning the SMCP, according to Shintani et al. (1995), the SMCP is not less accurate than the LSKP. In fact, SMCP seems superior to the LSKP with the proposed restriction (i.e., $n \geq 50$, $r \geq 1$, $r/n < 0.9$, where *n* is the number of BIs and *r* the number of negative BIs).

Due to its major simplicity, the half-cycle method is more popular than the fraction negative method and this last one more popular than the construction of the survivor curve (ISO 11135-1, 2007).

1.5. ETHYLENE OXIDE STERILISATION PROCESS OPTIMISATION

Optimisation of EO sterilisation processes is a challenge, due to the fact that the global competition market requires cost effectiveness, flexibility and inherent reduction of overall sterilisation process time, whilst continuing to comply with regulatory requirements and product quality (Sordellini et al., 2001; Strain and Young, 2004).

Traditionally, most of the EO sterilisation production time is taken up with two operations, which are when products are held waiting for the microbiological test results and/or for the validated aeration time to ensure residues levels in compliance with the requirements of ISO 10993-7. Implementation of parametric release simply eliminates the microbiological test phase from routine (EN 550, 1994; Sordellini et al., 2001; Allen, 2002; Strain and Young, 2004; ISO 11135-1, 2007).

Validation of not only sterilisation but also the aeration process, with consequent assessment of EO residues in compliance with the requirements of ISO 10993-7, has allowed a reduction in processing time. Furthermore, in order to get a reduction on EO residuals after sterilisation, research in the field of EO diffusion is required. Investigations about comparative efficiencies between different ways of carrying aeration are also lacking (Sordellini et al., 2001; Strain and Young, 2004).

In order to shorten production time, a joint study involving sterilisation and aeration processes limits shall be performed.

Direct measurement of key process variables, as well as improvements in process design achieved through scientific modelling and experimental evidence, allows development of each process phase and consequent reduction in an overall sterilisation process time.

EO sterilisation process optimisation will be explained below in more detail under three main topics: parametric release, lethality modelling and EO diffusion modelling.

1.5.1. Parametric release

Parametric release is the declaration of product sterilisation adequacy based solely on measurement and evaluation of physical process parameters compliant with previously validated parameters (EN 550, 1994; AAMI TIR 20, 2001; Strain and Young, 2004; ISO 11135-1, 2007).

The main difference between parametric and conventional release is the number of process parameters directly measured. According to EN 550 and ISO 11135-1 requirements, for conventional release compliance the parameters that should be directly measured are the time of each phase, the pressure throughout the process, and the head-space temperature. The remaining two critical parameters, humidity and EO concentration, can be quantified indirectly by thermodynamic calculation based on pressure rise and temperature. Acceptance of the two indirectly measured parameters is supported by the negative growth of the exposed BIs. According to conventional release procedure, the BIs data integrate and confirm that appropriate level of heat, water vapour and EO concentrations have been delivered to the load, as demonstrated during microbiological validation of the process (EN 550, 1994; Sordellini et al., 2001; ISO 11135-1, 2007).

The philosophy of parametric release is that once a cycle is validated, using direct analysis of all critical process parameters and distribution of microbiological indicators, the resulting data can be used to scientifically define the approved physical limits of each of the process parameters (EN 550, 1994; ISO 11135-1, 2007).

The parametric release is still a very updated issue, because the online methods for controlling and measuring physical parameters of EO sterilisation in the chamber, such as temperature, pressure, relative humidity and specially EO concentrations, were first reported in 2001 by HS Systems-und Prozesstechnik GmbH (Hucker and Axel, 2001).

Parametric release is from a scientific point of view, as well as from a strategic production standpoint, the preferred choice. In comparison with conventional release, the more thoroughly monitored process parameters give further understanding and a stricter sterilisation process control, simultaneously reducing costs and bringing a significant flexibility to the process (EN 550, 1994; Sordellini et al., 2001; Allen, 2002; Strain and Young, 2004; ISO 11135-1, 2007).

1.5.2. Lethality modelling

The mathematical modelling of the EO sterilisation cycle allows the definition of optimal inactivation conditions. The accurate prediction of D-values and process times, required for a target SAL, allows cycle times and/or EO concentration reduction as well as the comparison of effectiveness and equivalency of different sterilisation processes. Furthermore, the lethality modelling contributes to the process efficiency and flexibility, and the parametric release is much more scientifically supported (Brinston, 1995; Heider et al., 2002; Mosley et al., 2002).

Due to process complexity, EO sterilisation lethality is difficult to express mathematically. Many factors complicate the EO sterilisation process, such as: EO absorption; product material effects, due to reactions that can occur between the device and the sterilising agent; existence of microenvironments in the process chamber and; the achievement and maintenance of steady-state conditions. Furthermore, considering only the parameters

previously described for process design, such as EO concentration, exposure time, temperature and relative humidity parameters, the mathematical models for integrating all these variables and defining the EO sterilisation process lethality are still very recent (Rodriguez et al., 2001; Sordellini et al., 2001; Mosley et al., 2002b).

To integrate mathematically the dynamic temperature and concentration conditions effects on inactivation, Rodriguez et al. (2001) developed the following model for BI spores of *Bacillus subtilis niger*:

$$N(t) = \frac{N(t=0)}{e^{\left[k_{T_R}\int_0^t C(t)^n.10^{\frac{T(t)-T_R}{z}}dt\right]}} \quad (1.1)$$

where *N(t)*, *C(t)* and *T(t)* are the number of survivors, the EO concentration and the temperature at time *t*, respectively; k_{T_R} is the rate constant at a finite reference temperature T_R; *z* is the temperature increase required to reduce the decimal reduction time (D-value) by 90 %; *n* is a model parameter.

The model was validated under the following conditions: 15 to 90 % of RH, 200 to 1200 mg L^{-1} of EO, and z-value of 29.4 °C / 84.9 °F. The same authors also deduced an expression for determining the accumulated lethality of an EO sterilisation process (Rodriguez et al., 2001):

$$F_{T_R,C_R,z} = \frac{1}{C_R^n}\int_0^t C(t)^n 10^{\frac{T(t)-T_R}{z}}dt \quad (1.2)$$

where C_R is a reference EO concentration; *F* is the exposure time at T_R and C_R that would cause the same lethal effect as the *T(t)* and *C(t)* temperature conditions (i.e. equivalent process time).

Mosley et al. (2002b) deduced an alternative model for equivalent process time prediction:

$$F_{C_R,T_R} = \left(10^{\log t_{T_R}}\right)\frac{C}{C_R} \tag{1.3}$$

For varying EO concentration and/or temperature conditions, the equation would be:

$$F_{C_R,T_R} = \sum_{i=1}^{m} F_i = \sum_{i=1}^{m}\left(10^{\left[\log t_{T_i} + \frac{1}{z}(T_i - T_R)\right]}\right)\frac{C_i}{C_R} \tag{1.4}$$

where *i* is the process step in which the EO concentration and temperature are constant and *m* is the total number of process steps.

The mathematical models above presented are essential for designing EO sterilisation processes. Optimisation and validation of the different methodologies are a requirement.

1.5.3. Ethylene oxide diffusion modelling

The study of EO diffusion from the steriliser head-space to the articles and through the articles is a challenge matter. Once well understood and defined, efficiency comparison among different aeration methodologies, and prediction of the dynamic distribution of EO concentration within the load, with the purpose to define the specific relationship between EO concentration and process lethality as well as to diminish EO residuals after sterilisation, will be possible.

EO diffusion is influenced by several different extrinsic and intrinsic factors, respectively temperature, concentration, pressure and humidity, and, type, thickness, density, composition, and geometry of the packaging materials and article itself (Gibson et al., 1989; Andersen et al., 1997). The EO diffusion through the load of materials is also affected to a certain extent by a proportion of the EO molecules becoming immobilized, due to its "bounding" with constituents of the load.

The sterility assurance depends on sufficient concentration of EO being in contact with material surfaces within the load, but in contrast the EO concentration is monitored in the space outside the load/product, rather than within the load. Furthermore, the EO diffusion study through the materials is crucial for the definition of the specific relationship between EO concentration and process lethality (Matthews et al., 1998).

Considering permeable barriers, EO diffusion dynamics can be described by reference to Fick's first law of diffusion:

$$J = -D\frac{dC}{dx} \tag{1.5}$$

where *D* is the diffusion coefficient, *J* corresponds to the rate of diffusion and *x* to the distance.

Due to the EO and moisture load absorption, a decrease on the head-space levels happens. The packaging is an important factor, and according with Manning et al. (1997), each level of porous corrugated packaging and paper is capable of absorbing up to 10 % EO w/w. Consequently, the slowest permeation rate occurs at the primary packaging level. For a system where the product has several layers of packaging, the overall permeation time can be represented by the following equation:

$$\text{Total permeation time} = \sum \frac{kL^2}{D_i} \quad (1.6)$$

where *k* corresponds to a factor between 2 to 6; D_I is the diffusion coefficient in the layer *i* and *L*, the packaging layer thickness.

Gibson et al. (1989) developed a computerized model that seems to be more generally applicable than analytical methods in determining EO diffusion in polymeric materials.

However, despite the interest of this kind of studies, investigations in this field are almost inexistent. Once the modelling of accumulated lethality, as well as the modelling of EO diffusion according to different matrices is fully comprehended, new approaches for design, validation and routine control of the EO sterilisation processes will occur.

1.6. FINAL REMARKS

Ethylene oxide sterilisation is a key issue for current medical device designs, and considerable amount of work concerning this topic have been published. In terms of speed to the market, and despite the great advances already achieved, EO sterilisation and its unique capabilities are still in progress.

The research reviewed in this section allowed concluding about the necessity to understand the microbiological inactivation kinetics under EO sterilisation process conditions. In parallel, there is much to be done in order to understand EO diffusion behaviour on different materials. The ability to predict the dynamic distribution of EO concentration within the load being sterilised would allow the estimation of theoretical sterilising EO concentrations. Further developments are needed in terms of comparison of effectiveness and equivalency of different aeration methodologies.

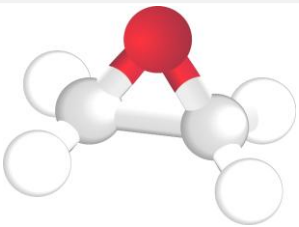
These topics will be addressed in the following chapters of the thesis.

Part I

Microbial lethality by ethylene oxide

Chapter 2

Modelling the inactivation of Bacillus subtilis spores by ethylene oxide processing



This chapter addresses predictive microbiology on ethylene oxide sterilisation. The effects and interactions of temperature, ethylene oxide concentration and relative humidity on the inactivation of the Bacillus subtilis, var. niger (ATCC 9372) were studied. Experiments were carried out using a full factorial experimental design at two levels (2^3 factorial design). Limit target exposure conditions for ethylene oxide concentration, temperature and relative humidity were 250 - 1000 mg EO L^{-1}, 40 - 60 °C and 50 - 90 %, respectively. Adopting a different approach from the first order kinetics, a Gompertz model was successfully applied in data fitting of the inactivation curves. Bacillus subtilis kinetic behaviour presented a sigmoidal inactivation with an initial shoulder (λ), followed by a maximum inactivation rate (k_{max}), these being model parameters. It was concluded that, temperature and ethylene oxide concentration were the most significant factors and consequently, additional experiments were carried out aiming at describing the parameters dependence on these process factors. Mathematical relations describing such dependences were successfully developed and included in the Gompertz kinetic model. The predictive ability of this integrated model was assessed, and its adequacy in predicting B. subtilis inactivation was proven.

2.1. INTRODUCTION

The main advantage of the ethylene oxide sterilisation methodology is its effectiveness and compatibility with most materials, as well as the flexibility of the EO sterilisation process, resulting from its dependency on several factors, such as concentration, temperature (T), humidity and time (and their combinations). Understanding the full dynamics of the sterilisation allows the definition of appropriate process variables, thus contributing to its design optimisation.

Bacillus subtilis, due to its resistance to EO sterilisation, is the most common microorganism used in the process control (ISO 11138-2, 2006). Heider et al. (2002) described a linear relation between the inactivation rate and EO concentration (in the range 50 to 1200 mg L^{-1}) and a similar tendency was observed for temperature (in the range 30 to 64 °C). The temperature and EO concentration were independent variables.

Relative humidity (RH) is a complex process variable and contradictory results were obtained by different authors (Oxborrow et al., 1983; Rodriguez et al., 2001; Mosley et al., 2002). Recent findings show that, within the limits of 30-90 %, the RH does not influence lethality (Rodriguez et al., 2001; Heider et al., 2002; Mosley et al., 2002; Mosley and Houghtling, 2005). Out of these limits, at very humid or dry environments, the diffusivity of EO into devices and microbes is compromised (Mosley et al., 2002; Mosley et al., 2002; Mosley and Houghtling, 2005).

The microbial inactivation by EO sterilisation has been considered to follow a first order kinetics, although model prediction is scarcely (or never) assessed. According to the first order kinetics approach, a plot of the logarithmic of the number of survival microorganisms vs. time is a straight line. The reciprocal of the slope is the well-known D-value (i.e. the required time for a 10-fold of the microbial load). However, a number of studies report deviations to the linear behaviour (Peleg, 2000; Juneja et al., 2001). To circumvent these situations, some authors excluded the initial shoulder period of the

experimental data (Shintani et al., 1995; Juneja et al., 2003) in data fitting, while others forget the convenient regression analysis procedures by performing linear regression to obvious non-linear experimental data patterns. Therefore, extrapolations of predicted times based on reported D-values from linear models, may not be appropriate.

Deviations from linearity can be assumed as a complete (or incomplete) sigmoidal behaviour with the following features: shoulder time (or lag) period, prior to a linear phase corresponding to a maximum growth/inactivation rate, followed by a tail (asymptotic phase). A number of models have been used to describe these sigmoidal tendencies (Sordellini et al., 1998; Chen, 2006): Weibull and logistic functions (Coleet al., 1993; Peleg and Cole, 1998; Buzrul and Alpas, 2004), Gompertz equation (Bhaduri et al., 1991), Baranyi and Geeraerd models (Baranyi and Roberts, 1994; Geeraerdet al., 2000).

Gompertz function, and its modified forms, has the ability of modelling both linear and asymmetrical sigmoidal data. For the inactivation behaviour, the following Gompertz function (Gibson et al., 1987; Bhaduri et al., 1991; Garthright, 1991; Linton et al., 1995; Chhabra et al., 1999; Gil et al., 2006; Kim et al., 2007) can be used:

$$\log\left(\frac{N}{N_0}\right) = A \cdot \exp\left[-\exp\left\{\frac{-k_{max} e}{A}(\lambda - t) + 1\right\}\right] \qquad (2.1)$$

where *N* is the microbial load at a particular process time *t* (the index *0* is related to initial microbial load). The model parameters are the maximum inactivation rate, k_{max}, and shoulder period, λ. The A value is the asymptotic response, being considered the tail (i.e. a resistant residual microbial population). It is not consensual whether the tail is actually a residual population or an enumeration method limitation. The Gompertz model is sometimes referred as being inadequate in predicting initial microbial load (i.e. for t=0, $log(N/N_0)$ only approaches zero). However, the over- or sub-estimation of this value may be negligible when compared to the experimental variations related to enumeration methods.

Studies on the influence of the process variables on microbial inactivation kinetics using ethylene oxide are lacking. Quantification of the kinetic parameters is obtained, as well as effect of relevant factors on their estimates. Consequently, the objectives of the present study were to: 1) screen the most significant variables on *B. subtilis* inactivation by EO sterilisation, 2) model the inactivation kinetics of *B. subtilis* including the variables' effects and, 3) provide a method of integrating lethality, thus contributing to the design optimisation and efficient control of the inactivation processes. This is certainly important when moving towards parametric release, i.e. the approval of the process relying merely on the measurement and assessment of process variables (ISO 11135-1, 2007).

2.2. MATERIALS AND METHODS

2.2.1. Experimental procedures

Experimental design

Experiments were carried out in an EO steriliser (please see *Sterilisation process* section) and conditions were defined according to a full factorial experimental design at two levels of three factors (2^3 factorial design; Box et al., 1978). The parameters used for each condition, including the time of each step, with the exception of the gas exposure time, were kept constant. Three independent variables representing temperature, EO concentration and humidity were assumed, each variable tested in two levels: a high level (+) and a low level (-), according to Table 2.1 (totalling 8 experimental conditions, corresponding to runs 1 to 8). Target limits for the exposure conditions of EO

concentration, temperature and relative humidity were 250 - 1000 mg EO L^{-1}, 40 – 60 °C and 50 - 90 %, respectively. The limits chosen for the process variables were based on literature review (Sordellini et al., 1998; Rutala and Weber, 1999; Heider et al., 2002; Mosley et al., 2002) and operating conditions of the sterilisers. However, difficulties arise in stabilizing the process conditions and actual attained operating values are in Table 2.1 *(all experimental data points are in Table B.1 - Appendix B)*.

The analysis of variance (ANOVA) allowed identifying the most significant parameters affecting microbial inactivation during EO sterilisation and, additional experiments considering intermediate conditions of these parameters were defined in order to model their effects and combined effects on the lethality (runs 9 to 15 included in Table 2.1).

Bacterial strain

Spore strip biological indicators (SGMStrip™, SGM Biotech, Inc., Montana, USA) were used in inactivation processes by EO. The biological indicators (BI) contain a known number of *B. subtilis* (ATCC 9372) spores (magnitude of 10^6 colony forming units (CFU), corresponding to an initial unprocessed microbial load, N_0) inoculated onto filter paper. Compliance with USP, ISO 11138 and EN 866 was verified.

The BI recovery technique was validated to obtain reliable spores enumeration. The recovery of spores from control samples (not submitted to the sterilisation process) was 70 % of the analysis report of the BI manufacturer.

Sterilisation process

All experiments were performed in a standard EO chamber (21059C Sterichem, France) of approximately 3 m^3 with controlled temperature, EO concentration and humidity. These conditions were maintained homogeneous inside the chamber due to forced recirculation, and were monitored by adequate equipment.

Temperature and relative humidity were monitored inside the load, using Kaye ValProbe® wireless data loggers, part numbers XVP-L-T and X2520, respectively. EO concentration was assessed by an infrared analyser in the steriliser chamber and corresponds to a condition obtained in the steriliser head-space, since the techniques currently available to the industry cannot measure this parameter inside the load, where the lethality of the process is being monitored.

For each of the defined exposure conditions, the sterilisation cycle was performed under vacuum, using a mixture of EO (Avantec, France) and nitrogen (Air Liquide, Portugal) with a minimum purity of 99.9 % and 99.5 %, respectively. EO was the sterilisation agent and nitrogen was applied to create a neutral atmosphere, avoiding flammable atmosphere inside the sterilisation chamber.

The sporicidal activity of a specific EO sterilisation cycle was assessed by placing the inoculated paper carriers with about 10^6 *B. subtilis* spores into the middle of peel-packs of surgical drape material (488-103, Bastos Viegas S.A., Portugal) adjacent to temperature and humidity sensors. BI samples were removed after different exposure times to the sterilising agent. Enumeration of the viable spores was performed according to the procedure described below.

Enumeration of viable spores

Enumeration of bacterial viable spores was performed in a laminar air flow cabinet (AH-100 Telstar®, Spain). Exposed spore carriers were transferred to a sterile screw cap, flat bottom tube with 6 glass beds of 5 mm containing 10.0 mL of sterile purified water (10 dilution) thus, the minimal detection limit is 10 CFU mL^{-1}. Tubes were vortexed for approximately 4 to 5 minutes (until the paper carrier is macerated to pulp) followed by a heat shock. Tubes were placed in a thermostatic bath (at 80 - 85 °C) for ten minutes and rapidly transferred into an ice bath (at 0 - 4 °C).

Samples were serially diluted and plated in triplicate onto tryptone soya agar (OXOID, UK). All plates were incubated at 32 ± 2 °C and counted after seventy-two hours (microbial load N).

The inactivation data was normalized in relation to initial load (please see *Bacterial strain* section) and expressed as logarithms (*i.e.* $log(N/N_0)$) *(results are included in Table B.2.1 to B.2.14 of Appendix B)*.

2.2.2. Modelling procedures

Equivalent time

Since standard-sized process chambers do not produce square wave cycles and significant lethality may occur throughout gas injection and exhaust phases, an equivalent exposure time, *U*, rather than exposure time, *t*, should be considered (in eq. 2.1). This is important since, the use of exposure time, which begins after steady-state pressure has been

achieved, rather than equivalent exposure time (*U*), leads to a gross underestimation of a process D-value, and a concomitant overestimation of the SLR (spore log reduction) and underestimation of the SAL (sterility assurance level).

The following expression can be used for the estimation of equivalent time (Mosley, 2002; Mosley et al., 2002b; Mosley and Gillis, 2004; Mosley and Houghtling, 2005):

$$U = \frac{t_{injection}}{2} + t_{nitrogen\ overlay} + t + \frac{t_{exhaust}}{2} \qquad (2.2)$$

assuming a constant rate of pressure increase (in the injection phase) and pressure decrease (in the exhaust phase).

Some authors (Mosley and Gillis, 2004; Mosley et al., 2005; Mosley and Houghtling, 2005) refer that the initial shoulder observed in the inactivation data is related to the consideration of the exposure time rather than the equivalent time.

Regression analysis and statistical assessment

The Gompertz model (eq. 2.1) was used to fit experimental inactivation data of $\log(N/N_0)$. The non-linear regression analysis was performed using the Levenberg-Marquardt algorithm to minimize the sum of the squares of the differences between the predicted and experimental values. Model parameters (i.e. k_{max} and λ) were estimated and their precision was evaluated by confident intervals by the standardised half width (SHW) at 95 %, i.e. halved confidence interval divided by the estimate ($SHW_{95\%} \equiv \frac{confidence\,interval_{95\%}}{2} \times \frac{1}{estimate} x100$).

The quality of the regression was assessed by residuals analyses (normality and randomness) and by the coefficient of determination R^2.

Results from 2^3 factorial experimental design were analysed by ANOVA procedures.

Statistica© 6.0 (StatSoft, USA), and Microsoft® Excel 2000 (Microsoft Corporation, USA) were used for all calculations and statistical analysis.

2.3. RESULTS AND DISCUSSION

2.3.1. Influence of environmental conditions on microbial inactivation kinetics

The influence of EO concentration, temperature and relative humidity on inactivation behaviour of *B. subtilis* spores was studied in a preliminary step using the conditions defined according to the 2^3 experimental design (runs 1 to 8 in Table 2.1). A slight deviation between the experimental and the target conditions occurred in some situations due to limitations of the industrial equipment. The shape of the inactivation curves (Figure 2.1) depends on the lethal agent intensity but, in general, significant deviations from linearity are evident and the general shape is a concave downward curve. As observed, the data exhibit an initial shoulder prior to exponential phase of death. Forcing a straight line through the experimental non-linear survival curves is obviously an undesirable option, and can lead to a considerable error of underestimation of process time when combined with extrapolation. The derived estimated times needed to obtain the target lethality seem to be higher than predictions derived from D-values calculations on "linear" inactivation curves and this should be avoided.

A Gompertz model (Gibson et al., 1987; Bhaduri et al., 1991; Garthright, 1991; Linton et al., 1995; Chhabra et al., 1999; Gil et al., 2006; Kim et al., 2007) was chosen due to its

versatility in describing different tendencies (from linear till pronounced sigmoidal shapes), depending on the magnitude of the model parameters. Results *(included in Table B.2.1 to B.2.7 of Appendix B)* showed that experimental inactivation data were successfully fitted with the Gompertz model *(results included in Table B.3.1 to B.3.7 of Appendix B)* and estimated parameters (i.e. λ and k_{max}) are included in Table 2.1. The goodness of model fitting was assessed on the basis of residuals randomness and normality (which was verified in all cases) and on the coefficient of determination (R^2 was greater than 0.98, meaning that at least 98 % of the observed variability was explained by the model).

Table 2.1 Estimated k_{max} and λ parameters of *B. subtilis* inactivation at the temperature, EO concentration and relative humidity conditions tested

Run	Variables						Parameters				Regression analysis
	T (°C)		C (mg L^{-1})		RH (%)		k_{max} x10^3 (s^{-1})	$SHW_{95\%}$(%)	λ (s)	$SHW_{95\%}$(%)	R^2
1	60	(+)	233	(-)	63	(-)	4.56	3.01	391.22	5.24	0.992
2	44	(-)	257	(-)	86	(+)	1.78	2.42	1079.26	3.25	0.993
3	34	(-)	222	(-)	60	(-)	0.989	2.93	1178.85	7.45	0.988
4	40	(-)	980	(+)	90	(+)	3.49	2.66	417.73	5.26	0.991
5	59	(+)	266	(-)	83	(+)	4.46	3.56	353.18	7.19	0.991
6	33	(-)	940	(+)	61	(-)	2.16	2.50	605.12	5.63	0.989
7	59	(+)	1004	(+)	98	(+)	7.65	8.59	265.92	16.75	0.983
8	60	(+)	977	(+)	46	(-)	10.00	*	0.00	*	*
9	37		674		73		2.28	2.72	831.31	4.05	0.991
10	37		456		80		1.83	2.57	821.44	4.68	0.991
11	51		247		80		3.23	3.60	481.61	7.10	0.985
12	51		447		67		4.09	3.37	300.04	8.67	0.994
13	50		675		72		5.04	4.94	256.72	14.53	0.992
14	60		738		71		7.33	8.01	254.67	17.83	0.994
15	62		498		77		5.89	6.25	291.40	12.25	0.988

* Meaningless value

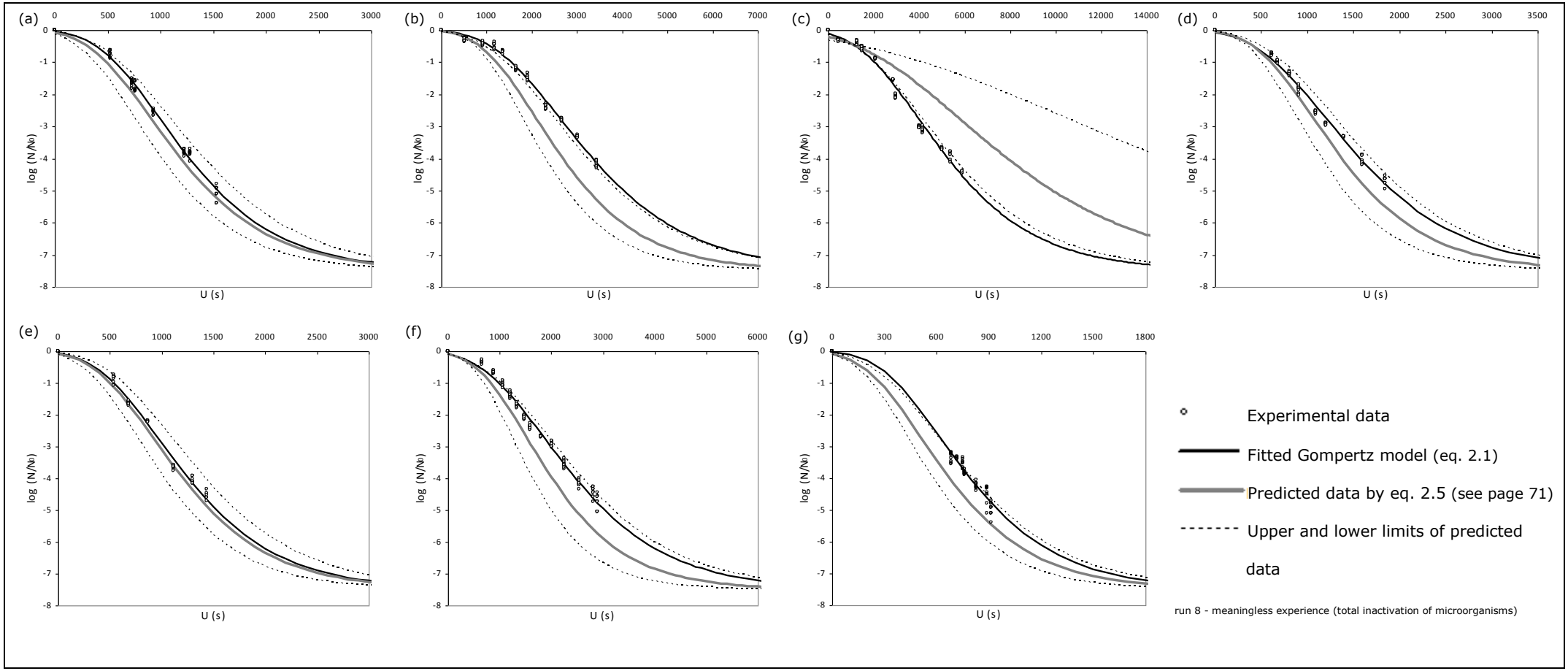


Figure 2.1 Inactivation of *B. subtilis* spores by EO sterilisation at conditions defined according to the 2^3 factorial design, (a) run 1, 60 °C, 233 mg L^{-1}, 63 %RH, (b) run 2, 44 °C, 257 mg L^{-1}, 86 %RH, (c) run 3, 34 °C, 222 mg L^{-1}, 60 % RH, (d) run 4, 40 °C, 980 mg L^{-1}, 90 %RH, (e) run 5, 59 °C, 266 mg L^{-1}, 85 %RH, (f) run 6, 33 °C, 940 mg L^{-1}, 61 % RH, (g) run 7, 59 °C, 1004 mg L^{-1}, 98 %RH

The overall inactivation curves were divided into a slower first stage (i.e., a shoulder phase) and in a second stage for exponential inactivation (i.e. maximum death rate). A true tailing was not observed under the conditions tested. The residual final value could be defined due to an enumeration method limitation and does not correspond to a residual resistant population. Consequently, the tail was not defined as a model parameter, and an asymptotic value of -7.5 was assumed (reflects overall tendency in all experiments and avoids interference with the studied kinetic parameters).

High precision of k_{max} and λ estimates was attained (evaluated by $SHW_{95\%}$). The poor results were obtained for conditions of run 7 with a standard half width of 8.6 % for k_{max} (the remaining values averaging 2.8 %) and 16.8 % for λ (the remaining values averaging 5.7 %). For both, λ and k_{max}, higher errors tend to be obtained for the highest temperatures and EO concentration. This is reasonable since, for a higher lethal condition, the inactivation is faster and, consequently, there is a lack of experimental points, mainly in the initial period, which implies lower precision in the estimation of parameters.

The factorial experimental design allowed concluding about the process variables (and combination of them) that significantly affected the inactivation kinetics of *B. subtilis* behaviour (assessed by k_{max} and λ parameters). Results showed that temperature had the most significant effect on k_{max} and λ, followed by EO concentration (at a significance level of 15 %). Therefore, special attention should be given to the accuracy of the temperature sensors used to monitor the sterilisation conditions. The temperature and the EO concentration have a negative effect on λ and a positive effect on k_{max}. This means that higher temperatures and EO concentration imply narrow shoulder times and higher inactivation rates. On the contrary, lower inactivation rates and more evident shoulder phases were observed at the lowest temperature and EO concentration. Effects resulting from the combination of temperature and EO were not significant. Also, the relative humidity (and its combined effects with the remaining variables) did not influence

significantly the inactivation of *B. subtilis (ANOVA results included in section B.4 of Appendix B).*

Based on these achievements, seven additional experimental conditions were tested using intermediate conditions of temperature and concentration, combined with the limits previously defined: one temperature (about 50 °C) and two more EO concentrations (about 470 mg L^{-1} and 700 mg L^{-1}). These experiments provided important data *(included in Table B.2.8 to B.2.14 of Appendix B)* aiming at describing more accurately the dependence of kinetic parameters on environmental conditions. These results are also included in Table 2.1 (runs 9 to 15) and inactivation curves in Figure 2.2 *(results included in Table B.3.8 to B.3.14 of Appendix B)*. Model adequacy and goodness of fits were assessed as previously mentioned. A good precision of parameter estimates was observed, since low errors were attained (maximum $SHW_{95\%}$ values of 8.0 % and 17.8 %, respectively for k_{max} and λ, again for the highest variables values, with the remaining value averaging 3.9 % and 8.5 % for k_{max} and λ, respectively).

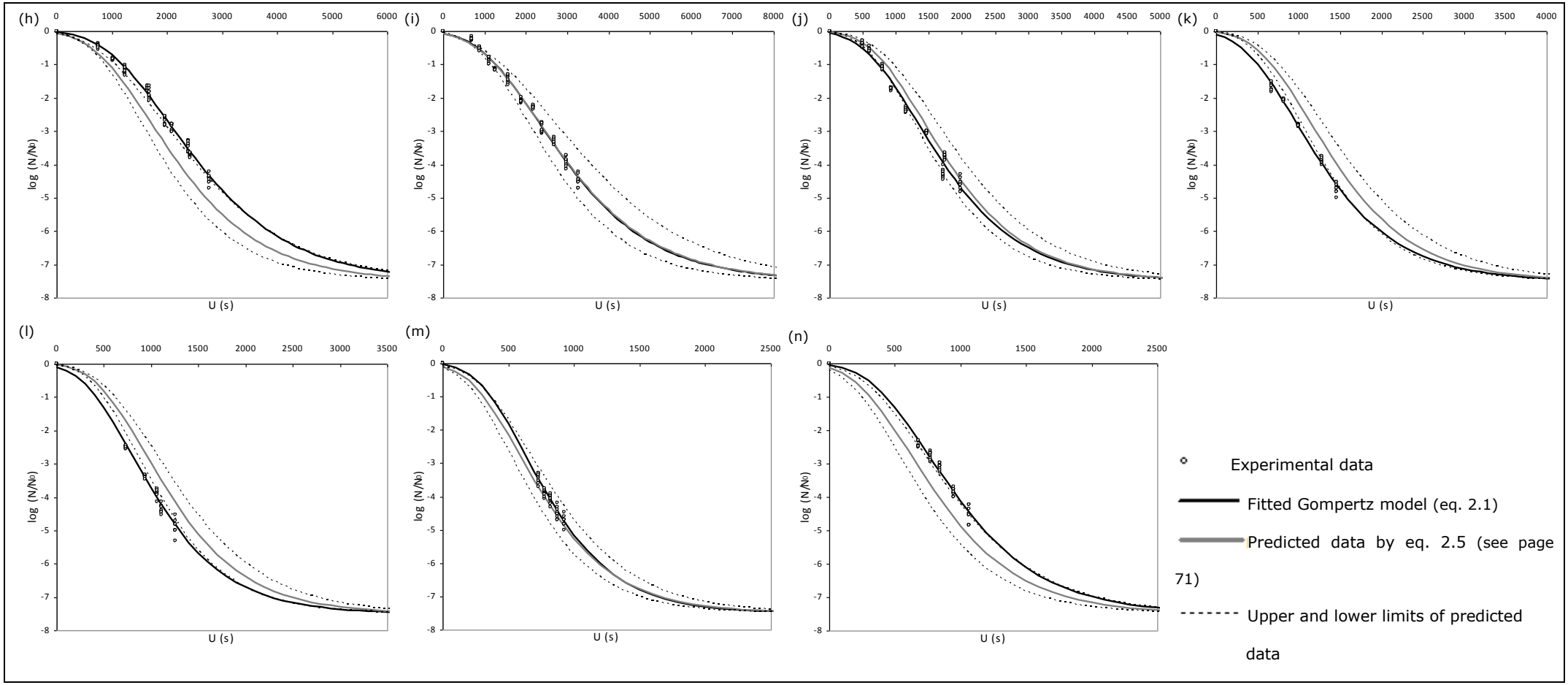


Figure 2.2 Inactivation of *B. subtilis* spores by EO sterilisation at conditions defined according to the 2^3 factorial design, (h) run 9, 37 °C, 674 mg L^{-1}, 73 %RH, (i) run 10, 37 °C, 456 mg L^{-1}, 80 %RH, (j) run 11, 51 °C, 247 mg L^{-1}, 80 %RH, (k) run 12, 51 °C, 447 mg L^{-1}, 67 %RH, (l) run 13, 50 °C, 675 mg L^{-1}, 72 %RH, (m) run 14, 60 °C, 738 mg L^{-1}, 71 %RH, (n) run 15, 62 °C, 498 mg L^{-1}, 77 %RH

Regarding temperature, Q_{10} is the coefficient that defines the change in the reaction rate constant for a change in temperature of 10 °C. The reported Q_{10} for destroying spores with EO has been reported to be in the range of 1.4 to 2.0. A consensus seems to consider a value close to 2 for Q_{10}, which means that a 10 °C change would affect lethality by a factor of 2 and the z-value would be very close to 30 °C (Richardson and Hyslop, 1985; Demitrius et al., 1993; Heider et al., 2002; Mosley et al., 2002; Mosley et al., 2002).

If one considers the pairs of runs with identical EO concentrations (i.e. runs 1-11, 15-12, and 14-13) and temperatures differing by 10 °C (i.e. sterilisation temperatures of 60 °C and 50 °C, respectively), the inactivation rate differs by a factor of 1.4. However, if other runs are compared (i.e. runs 2-3 at 44 °C and 34 °C, respectively), the value obtained is 1.8.

2.3.2. Assessment of model prediction

Temperature and EO concentration were the most significant factors affecting *B. subtilis* inactivation parameters. Consequently, equations that describe these influences on k_{max} and λ were developed aiming at obtaining a mathematical inactivation model expressed in terms of the relevant processing variables. It was assumed that k_{max} varied linearly with EO concentration, for given temperatures ($k_{max}=a_k+b_kC$). Some authors had reported a plateau effect in the inactivation rate within the range 250 – 1000 mg L^{-1}, at higher ethylene oxide concentrations, but Mosley et al. (2002, 2005) did not observed such effect. This last finding is in accordance with our results (Figure 2.3a). Concerning λ parameter, the relation $\lambda=a_\lambda+ b_\lambda \ln C$ provided the best fits (Figure 2.3b).

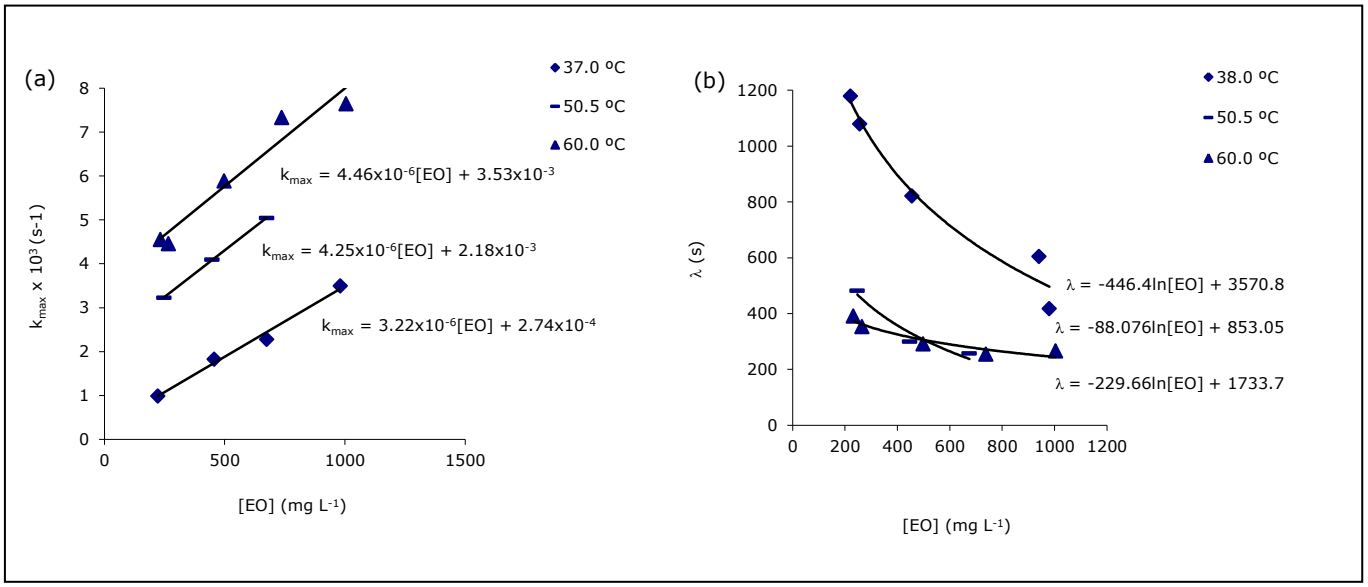


Figure 2.3 Influence of EO concentration on kinetic parameters, k_{max} and λ

(a) Influence of EO concentration on k_{max}

(b) Influence of EO concentration on λ

The parameters a_k and b_k, as well as a_λ and b_λ, were estimated by regression analysis procedures, and the influence of temperature on these estimates were studied. Linear relations of these parameters on temperature were defined (Figure 2.4). The quality of the model fits were attained by residual analysis and R^2 magnitude, that in all cases were above 0.90.

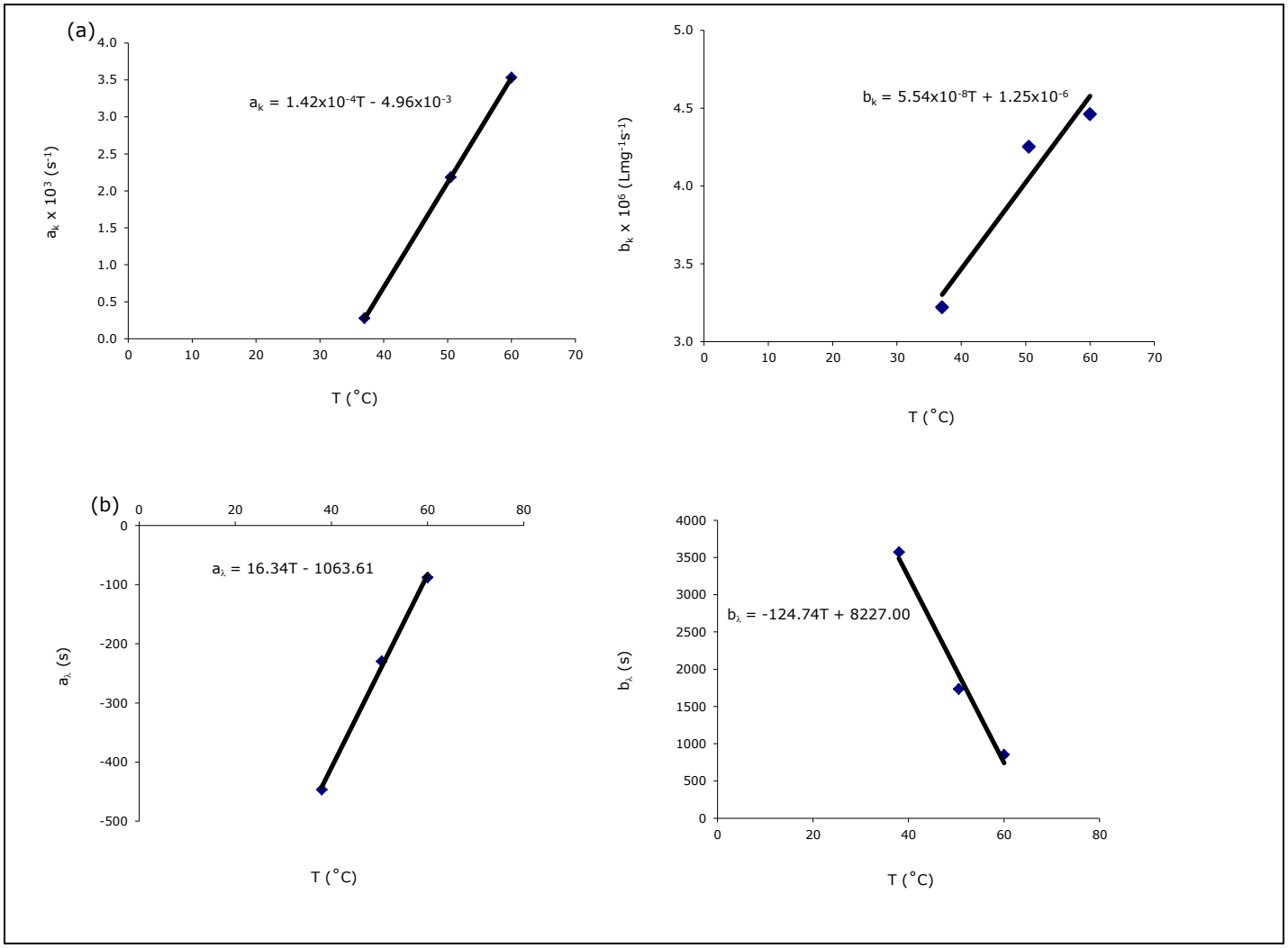


Figure 2.4 Influence of T on a_k and b_k (Figure2.4a) and a_λ and b_λ (Figure2.4b) parameters

Merging all the developed equations, the final expressions that relate k_{max} and λ with temperature and EO concentration are the following:

$$K_{max} = \left(1.42x10^{-4}T - 4.96x10^{-3}\right) + \left(5.54x10^{-8}T + 1.25x10^{-6}\right)C \quad (2.3)$$

$$\lambda = \left(1.63\times10^{1}T - 1.06\times10^{3}\right)\ln C + \left(-1.25\times10^{2}T + 8.23\times10^{3}\right) \quad (2.4)$$

The predictive ability of these expressions were assessed by plotting the estimated values of k_{max} and λ based on experimental data fits using the Gompertz model (eq. 2.1) *versus* the ones obtained using the previous equations (Figure 2.5 and Table 2.2). It can be concluded that for k_{max}, eq. 2.3 allows a good prediction (a strong agreement between k_{max} estimated on the base of experimental data and the one expressed in terms of processing variables). Concerning λ, eq. 2.4 allows a satisfactory prediction. It was observed a higher dispersion of λ estimated on the base of experimental data and the one expressed in terms of processing variables, when compared with the results obtained for the inactivation rate. This is not unexpected, since the shoulder parameter is estimated with less precision (results previously discussed).

Table 2.2 Estimated and predicted k_{max} and λ parameters of *B. subtilis* inactivation

Run	Parameters			
	k_{max} x10^3 (s^{-1})	k_{max} x10^3 (s^{-1}) pred. †	λ (s)	λ (s) pred. ††
1	4.56	4.55	391.22	307.1
2	1.78	2.18	1079.26	837.9
3	0.989	0.61	1178.85	1231.2
4	3.49	4.18	417.73	409.8
5	4.46	4.57	353.18	321.8
6	2.16	2.69	605.12	516.2
7	7.65	8.04	265.92	174.0
9	2.28	2.52	831.31	621.9
10	1.83	1.80	821.44	801.2
11	3.23	3.29	481.61	596.6
12	4.09	4.06	300.04	467.0
13	5.04	4.84	256.72	384.9
14	7.33	6.95	254.67	191.8
15	5.89	6.17	291.40	179.8

† Eq. 2.3

†† Eq. 2.4

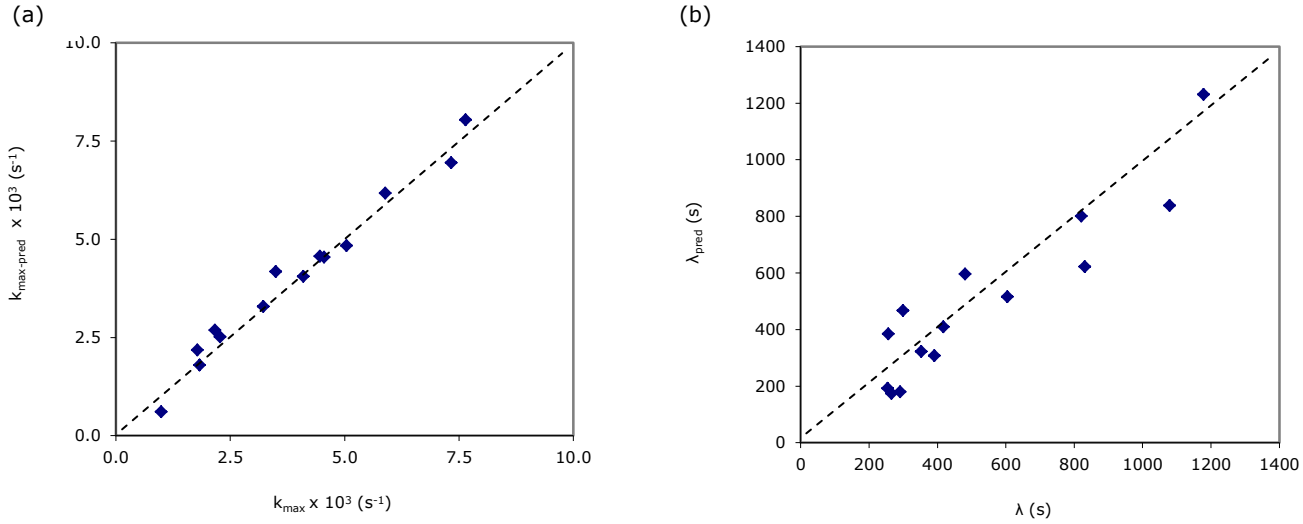


Figure 2.5 Inactivation rate, k_{max} (Figure2.5a) and shoulder, λ (Figure 2.5b) parameters estimated by the Gompertz model (eq. 2.1) *versus* predicted values as a function of temperature and EO concentration (eq. 2.3 and 2.4, respectively)

The final objective of this work was to express the inactivation data (i.e. $\log(N/N_0)$) in terms of the most significant processing variables (i.e. T and EO concentration). Consequently, eq. 2.3 and 2.4 were integrated in the Gompertz model (eq. 2.1). The final expression was:

$$\log\left(\frac{N}{N_0}\right) = (-7.5)\exp\left\{-\exp\left[\frac{-\left[\left(1.42x10^{-4}T - 4.96x10^{-3}\right) + \left(5.54x10^{-8}T + 1.25x10^{-6}\right)C\right]e}{-7.5}x \right.\right.$$
$$\left.\left.x\left[\left(1.63x10^{1}T - 1.06x10^{3}\right)\ln C + \left(-1.25x10^{2}T + 8.23x10^{3}\right) - U\right] + 1\right]\right\} \quad (2.5)$$

Or deducted for *U*:

$$U = \lambda - \frac{(-7.5)}{k_{max} \times e}\left\{1 - \ln\left(-\ln\left(\frac{SAL}{(-7.5)}\right)\right)\right\} \quad (2.6)$$

where *SAL* is the sterility assurance level and *C* corresponds to the EO concentration in the steriliser head-space chamber. As already discussed, the tail parameter was assumed to be -7.5.

Once the diffusivity for this specific load is achieved, it would be possible to re-adjust this equation and replace this parameter by a general one that corresponds to the EO concentration where lethality of the process is being monitored. This effort would be more profitable if simultaneously, equipment is developed to measure the EO concentration inside the load.

The prediction of *B. subtilis* inactivation by the newly developed model can be visualized in the Figures 2.1 and 2.2 (*results included in Tables B.3 of Appendix B*). The grey line was obtained considering the average values of temperature and EO concentration. One should be aware about difficulties in reproducing (and/or stabilizing) experimental conditions. Based on experimental results, it was found a variability of 4 % for temperature and 14 % for EO conditions. These allow considering that the temperature and EO concentration changes throughout all cycles are not significant and constant conditions may be assumed without compromise lethality prediction.

The predictive ability of the final model was assessed considering a band of prediction (upper and lower limits defined by considering the maximum fluctuations of temperature and EO concentration and calculated using eq. 2.5).

The study outlining the sensitivity of the predicted microbial response in relation to processing factors (temperature and EO concentration) is included in *section B.5 in*

Appendix B. These bands (Figures 2.1 and 2.2) include the experimental data *(included in section B.6 in Appendix B)* for all the conditions tested, which demonstrates that the inactivation of *B. subtilis* under EO sterilisation can be successfully predicted using a model that only takes into account the process variables.

Although all efforts have been done in order to reproduce the experimental conditions of the different runs (similar industrial cycle design), the steriliser does not operate as a *B*iological *I*ndicator *E*valuator *R*esistometer (BIER) vessel. Due to the dimension of this type of equipment (BIER), it generates reproducible results through reliable process control and monitoring, as well as a square wave EO sterilisation cycle is attained, that would reduce the error related to the integrated lethality. In the steriliser, the EO concentration corresponds to a condition obtained in its head-space and not to the one inside the load (as explained on *Materials and methods – Sterilisation process*). The accuracy associated to the equipment used for monitoring the temperature and EO concentration also contributes to the overall predictive uncertainty (*results included in Appendix B*).

2.4. CONCLUSIONS

The *B. subtilis* EO inactivation did not follow a first order kinetics (i.e. linear inactivation). Experimental data showed an initial shoulder and a maximum inactivation rate period, and a Gompertz model was successfully applied in data fitting. The most relevant process variables that significantly affected the kinetic parameters were the temperature and the EO concentration. Mathematical models that describe the dependence of shoulder and maximum inactivation rate on such variables were also developed. These expressions were merged into the Gompertz model and accurate predictions of the original inactivation data were attained. Overall, an inactivation model expressed only in terms of the relevant process variables was achieved. The conventional design of EO sterilisation cycles usually involves a significant amount of experimental work, which is time consuming and also expensive. The results of this work are certainly a contribution for an efficient control, design and optimisation of the EO sterilisation process.

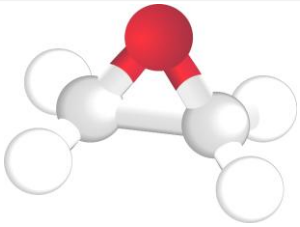
Despite the inexistence of standard guidelines on this topic, the predicted times for a target lethality should not be confined to D-values calculation, since non-linear tendencies occur.

Part II

Ethylene oxide transport phenomena

Chapter 3

Design and development of an apparatus for measuring ethylene oxide permeability of sheet materials – a case study



The gas permeability of sheet materials is very useful in many industrial domains and understanding ethylene oxide (EO) sterilisation effectiveness is one of the applications. A permeation apparatus was designed, conceived and developed for the measurement of ethylene oxide transport properties. The goal was to determine EO diffusion coefficient, solubility, and permeability of sheet materials by lag time methodology.

The operation of this apparatus, usually designated as permeation cell, is based upon the principle of measuring a transient change in pressure at conditions of constant volume and temperature, which allows the estimation of the steady state rate of gas transfer through membranes.

The type of permeation cell herein described has been previously published and accurate and reproducible measurements of mass transfer coefficients through different matrices have been attained.

3.1. INTRODUCTION

The ethylene oxide (EO) sterilisation effectiveness is related with the chemical permeation characteristics of materials and medical devices because the lethality was found to be directly correlated to the concentration of sterilant. Therefore, this property can be used for understanding, prediction and optimisation of the sterilisation and aeration processes (Yeom et al., 2000). This dichotomy is the key of the overall process optimisation and these studies are useful in providing an overview of the nature of EO transport in medical devices and packaging materials.

In the past, several techniques have been developed for determining the transport parameters related to polymeric membranes, and they have been discussed in an extensive review by Lomax (1980a, b). Among such experimental methods, and contrasting with the gravimetric methods, two have received particular attention: (i) the constant-pressure, variable-volume (volumetric technique), and (ii) the constant-volume, variable pressure methods (manometric, barometric or isochoric technique) (Flaconneche et al., 2001; Tremblay et at., 2006). According to the variable-volume method, a high pressure of gas is introduced into a permeation cell with the membrane under investigation and the gas to be studied is allowed to expand against a low constant pressure. The flow is obtained by measuring the change in volume of the permeate chamber. In the variable-pressure method, both sides of the membrane are initially under vacuum; the gas is then introduced in one side of the membrane, the surface concentration will remain constant and the rise in pressure in the opposite side is monitored as a function of time. These two methods have been compared by Stern et al. (1963), concluding that, for thin membranes, results obtained from both methodologies were in good agreement.

The variable-pressure method has been extensively used for several decades and today corresponds to the most used technique for measuring permeation through membranes. It has been an elected method, because it is well suited and more precise for measuring the permeability of any gas separation membrane, including films with small permeation rates. By knowing the reception volume as well as the operating conditions, the pressure signal is converted in a membrane flow, allowing calculations of the permeation rate.

The variable-pressure technique was firstly described by Schumacher and Ferguson (1927) to measure diffusion coefficients; it was later used by Barrer (1939) and Meares (1954) to determine permeabilities. Pye et al. (1976) described a system which was much more "user friendly", because it eliminated the use of mercury as a vacuum seal (ASTM D1434, 1982).

A number of commercial apparatus for measuring the permeability of polymeric films are available and all operate on similar basic principles. Generally, the equipments are complex, expensive and specific to certain standard permeants. However, and due to the interest in determining the transport parameters of a very specific gas, ethylene oxide, it was designed and conceived an inexpensive apparatus for experimental determination of gas diffusivity, permeability and solubility in sheet materials, using a variable-pressure approach by lag time methodology (following described).

A detailed description and assessment of the apparatus to measure the lag time and steady state flow in polymers from a single experiment is presented in section 3.2.2.

3.1.1. Gas permeation theory

The permeability of gases in polymers is a property inherent to their structure, resulting from the absorption of fluids by the material and from its diffusion through the polymer

matrix. The models describing the random molecular motion of gases through polymeric membranes are based on a solution–diffusion mechanism (*i.e.* three-step mechanism) (Geankoplis, 1983;Flaconneche et al., 2001 ; Tremblay et al., 2006).

This process comprises three steps: (i) sorption (adsorption or absorption) at the upstream boundary, (ii) diffusion through the membrane and (iii) desorption at the downstream side of the membrane. The driving force behind the transport phenomenon involving sorption, diffusion and permeation is the gradient of the concentration between the membrane sides (Crank, 1975). Fick's first law of diffusion is descriptive of such processes when the steady state of flow is attained. Accordingly, the flow (*J*), i.e., the amount of gas permeant passing through the polymer film per unit area and time, is proportional to the concentration (*C*) gradient, measured normal to any position (*x*) through the membrane (Crank, 1975; Geankoplis, 1983; Singh, et al., 1998 ; Friess, et al., 2004; Tremblay et al., 2006):

$$J = -D\frac{dC}{dx} \tag{1.5}$$

Considering a plane sheet, and if the concentration at both sides of the membrane is constant, the previous equation is equivalent to:

$$J = D\frac{C_u - C_d}{l} \tag{3.1}$$

where the indexes *u* and *d* corresponds to the upstream and the downstream side of the membrane, respectively, and *l* is the membrane thickness.

The concentration may be replaced by the gas partial pressure gradient, if an ideal gas is under study:

$$J = \mathscr{P}\frac{p_u - p_d}{l} \tag{3.2}$$

where $\mathscr{P}$ is the permeability coefficient.

When the downstream pressure is much lower than the upstream pressure, the molecule sorption is typically described by Henry's law and if the diffusion coefficient is constant over the pressure range, the solubility coefficient, *S* is obtained (Tremblay et al., 2006):

$$S = \mathscr{P}/D \tag{3.3}$$

3.1.2. Lag time method

In this method, the permeant gas accumulates in a pre-evacuated downstream volume. Under these conditions, before reaching the steady state, the flow rate and gas concentration into the membrane vary with time (Heilman et al., 1956; Yeom et al., 1999; Tremblay et al., 2006). If essentially zero concentration is maintained at the low pressure side of the membrane and the concentration of the gas in the first layer of the membrane at the high pressure side is held constant, a pressure difference almost constant is obtained. In addition, if the permeation time is high enough, then steady state is achieved and the curve of the amount of gas permeating through the membrane, *Q*, against time, *t*, tends to be a straight line described by Fick's second law of diffusion

(Heilman et al., 1956; Crank, 1975; Lin and Freeman, 2004; Lin and Freeman, 2006; Tremblay et al., 2006):

$$Q = \frac{DC_0}{l}\left(t - \frac{l^2}{6D}\right) \tag{3.4}$$

When *Q* is equal to zero, the intercept on the *t*-axis corresponds to the lag time, θ:

$$\theta = \frac{l^2}{6D} \tag{3.5}$$

The variable *l* represents membrane thickness, and *D* is the diffusion coefficient.

Thus, determination of θ leads to the evaluation of the diffusion coefficient, *D*, from eq. 3.6 while determination of the slope of the straight line leads to the permeability constant, $\mathscr{P}$, from eq. 3.3. Finally, the solubility coefficient, *S*, can be obtained from eq. 3.4 (Crank, 1975).

The standard mathematical analysis underlying the lag time technique assumes that the diffusion coefficient is constant throughout the permeation process from initiation to steady state permeation (Watson and Baron, 1995; Yeom et al., 1999; Yeom et al., 2000).

Care should be taken to high-diffusivity films, since the steady state is established too quickly to obtain an accurate value of the lag time (Heilman et al., 1956). Depending on the chemical and physical structure of the polymer and of the permeant, temperature, humidity and permeant concentration, the steady state may be attained in a few seconds or in days.

An example of a typical experimental measurement for gas permeation is schematically shown in Figure 3.1.

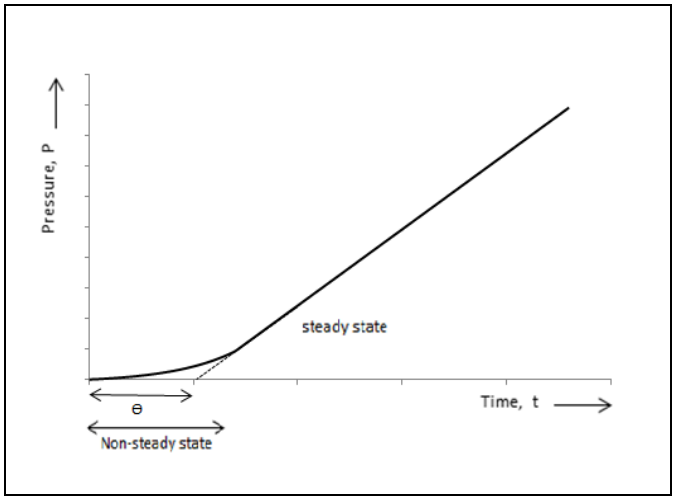


Figure 3.1 Plot of pressure versus time

The response curve is divided into two regions: (i) the first corresponds to the initial transient rise in pressure, which is designated as the non-steady state diffusion region, and (ii) the second is designated as the steady state diffusion, and corresponds to the linear portion of the response.

When measuring the permeability coefficient, it is essential that steady state gas transfer occurs. This is achieved when a constant gradient is obtained across the film, or said in another way, when equal quantities of permeant enter and leave the polymer, this corresponding to the near linear (steady state) part of the pressure versus time curve.

The lag time is the amount of time required for a gas (or vapour) to permeate through a membrane. Therefore, it is defined where pressure is zero on the time axis, when a line is extrapolated to the time axis from the linear part of the response curve.

The design and operation of the developed apparatus allows the determination of the diffusion coefficient by lag time methodology, through measure of the permeation transient and permeability coefficient, considering the flow at steady state conditions (Watson and Baron, 1995; Tremblay et al., 2006).

3.2. MATERIALS AND METHODS

3.2.1. Materials

The sample used in this study corresponds to the laminate of the surgical drape material (488-103, Bastos Viegas S.A., Portugal), composed by a polyethylene film bonded by a hotmelt adhesive to a viscose non-woven, the same material as the one used for modelling the inactivation of *Bacillus subtilis* (Chapter 2).

The test specimens were cut to fit the test cell (circular appropriate size), as described in section 3.2.2. The thickness of the samples were measured with a micrometre (TMI 49-63 Micrometre, TMI, USA) and were obtained considering the average value of five points distributed over the entire test area (prior to placement of the specimen in the equipment, as well as after the experiment, in order to confirm that there is no expand or contract during mass transfer). Membrane thickness is 188 ± 12 μm.

The permeant gas is ethylene oxide (Avantec, France) with a minimum purity of 99.9 %. It was fed from a 50 L cylinder (pressurized with nitrogen at 4 Bar) and vaporized in an industrial heat exchanger (from Bastos Viegas EO sterilisation unit).

3.2.2. General method

Permeation cell

A closed, sealed cell was constructed such that the sheet material to be tested was held sandwiched between two static half-cells, receiving and source chamber (Figure 3.2). The gas diffusion to be evaluated (ethylene oxide) was spiked to one side of the material under test (source chamber) in which constant high pressure was kept. The pressure increase was monitored on the opposite side of the material (the receiving chamber).

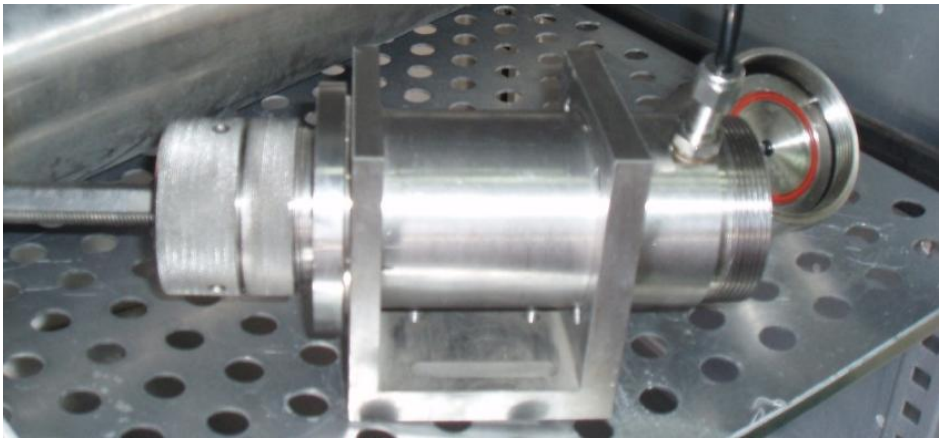


Figure 3.2 Permeation cell

The cylindrical cell was constructed of stainless steel and the specimen under test was placed between two rubber O-rings, clamped on a stainless steel screen and supported by a stainless steel highly microporous sintered disc. A vacuum-tight closure and a gas-tight seal were ensured by the design construction of the cell.

The cell is suitable for most gas-membrane systems, because the downstream volume can be regulated. This means that the volume of chamber can be changed according to the materials being tested (higher volumes for highly permeable materials and conversely, lower volumes for materials of low permeability).

General apparatus

A schematic representation of the experimental setup developed is shown in Figure 3.3.

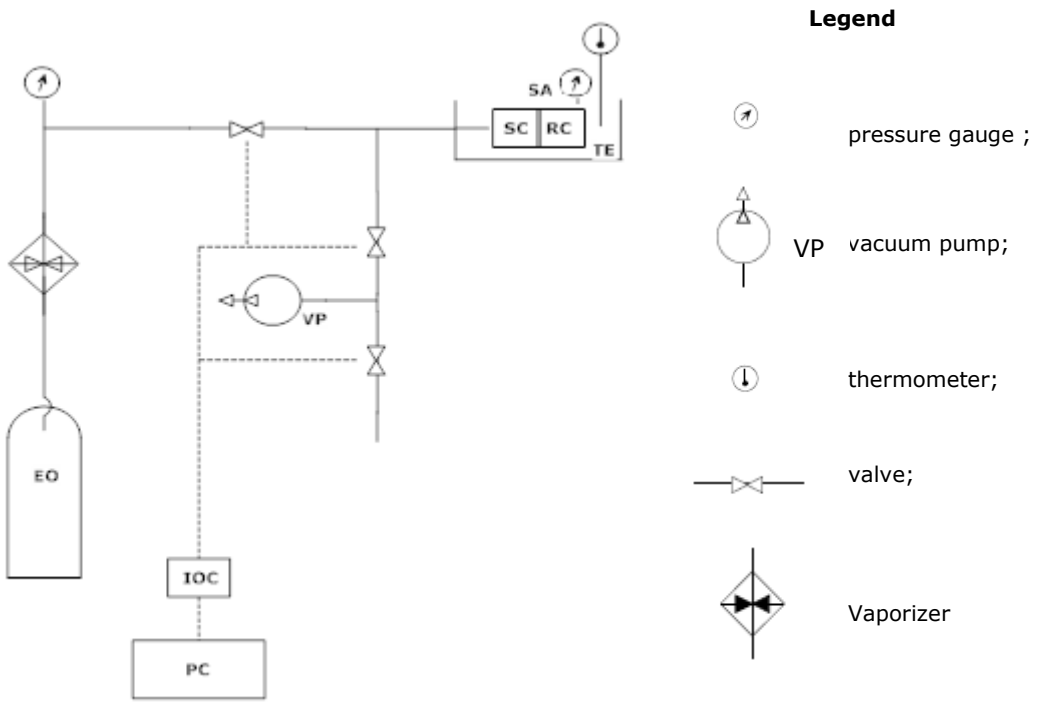


Figure 3.3 Experimental setup of the apparatus for measuring gas permeability: (EO) EO cylinder; (PC) personal computer; (RC) receiving chamber (of permeation cell); (SC) source chamber (of permeation cell); (SA) sheet sample; (TE) thermostated environment;

The apparatus consists of a permeation cell connected to a high vacuum system achieved by a oil vacuum pump, capable of reducing the pressure in the system to less than 26 Pa (model R5 0025 E, BUSCH, USA). A pressure sensor with digital indicator (CPH 6200, with a range from 0 to 2.5 Bar, WIKA, USA) and a pressure gauge (213.53.100, with a range from 0 to 5 Bar absolute, WIKA) were used to measure the pressure in downstream and upstream half-cell, respectively. The pressure supply of ethylene oxide was maintained constant by means of a gas pressure regulator. The electronic valves (SCE 272A 0.52 MS and 43005099, ASCO, USA) were actuated following a sequence determined by the experimental procedure.

The permeation cell was placed inside the heating oven (1600 HAFO SERIES 1685, VWR, USA), so that the experiments were performed at an imposed and constant temperature of 48 ± 0.1 °C.

Operation of permeation apparatus

The experimental method used to measure the rate of EO transmission is essentially similar to the high vacuum technique of Barrer, a classical method (manometric method) of studying permeation through polymer films and membranes (Heilman et al., 1956; ASTM D1434, 1982).

The sample sheet was placed in the apparatus so as to form a sealed semi-barrier between two chambers, and the entire system was evacuated and degassed until a vacuum of about 26 Pa. Evacuation of downstream volume up to high degree of vacuum is necessary for an accurate measurement of the permeation. After adapting the sample, both upstream and downstream volumes (including the membrane) were exposed to vacuum. After closing off the vacuum line, the leak test was done for at least one day,

before performing permeation experiments. The tightness of the system was always measured before permeation experiments; it should be at least ten times lower than the estimated steady state rate of pressure rise due to permeation.

After confirming leak absence, the gas was introduced into the cell at a fixed and constant pressure, as recorded by the pressure gauge. The feed pressure was maintained virtually constant during the measurements, so the upstream side of the film was exposed to a fixed pressure of penetrant.

The ratio of EO volume initially available and the volume of gas transferred at the end of the test shall be at least 100:1 (ASTM D1434, 1982).

The low downstream pressure coupled with a (virtually) constant feed pressure of 3.2 ± 0.2 Bar allows the assumption of a constant pressure difference across the membrane (during the measurements).

The gas flow rate through the specimen was monitored by continuously measuring the pressure increase on the pressure gauge connected to the low pressure half of the cell.

The whole process was repeated three times, using the same material (3 replicates).

A software specifically developed for control of the system (valves control) and data acquisition (from pressure sensor) recorded the pressure increase throughout all process.

Mathematical approach

The permeability of ethylene oxide through the membrane was determined by a classical constant-volume variable-pressure permeation method and the expression for calculation of the gas permeability is:

$$\mathscr{P}_A = \frac{V_d l}{P_u A R T}\left[\left(\frac{dP_d}{dt}\right)_{ss} - \left(\frac{dP_d}{dt}\right)_{leak}\right] \quad (3.6)$$

Where V_d is the downstream volume (m^3), l is the film thickness (m), P_u is the upstream absolute pressure (Pa), A is the film area available for gas transport, also named effective membrane area (m^2), the gas constant R is 8.314472 $m^3PaK^{-1}mol^{-1}$, T is absolute temperature (K) and $(dP_d/dt)_{ss}$ and $(dP_d/dt)_{leak}$ are the steady state rates of pressure rise (Pa s^{-1}) in the downstream volume at fixed upstream pressure and under vacuum, respectively (ASTM D1434, 1982; Czichos et al., 2006; Lin and Freeman, 2006).

The diffusion coefficient, D, can be determined by the lag time technique and obtained by eq. 3.6. From the slope of the straight line, using the steady state flow, the permeability coefficients, $\mathscr{P}$ (expressed in mol s^{-1} m^{-1} Pa^{-1}) can be calculated with eq. 3.7. When the diffusion coefficients and the flows in steady state are known, the solubility coefficient, S (expressed in mol m^{-3} Pa^{-1}), can be obtained by eq. 3.4.

3.3. RESULTS AND DISCUSSION

The transient flow curves (plot of the pressure changes versus time, t-t_0), obtained by the permeation apparatus, allowed to determine: (i) the permeability coefficient from the steady state rate of pressure rise in the downstream receiving volume (based on the construction of the best straight line through the points), and (ii) the diffusion coefficient using the lag time equation, considering the non-linear pressure rise developed just as the curve departs from almost zero, prior to its linear growth (Watson and Baron, 1995; Tremblay et al., 2006).

The plotting of the pressure (permeant gas pressure) *versus* time for samples of laminate is in Figure 3.4 (three replicates; runs 1 to 3).

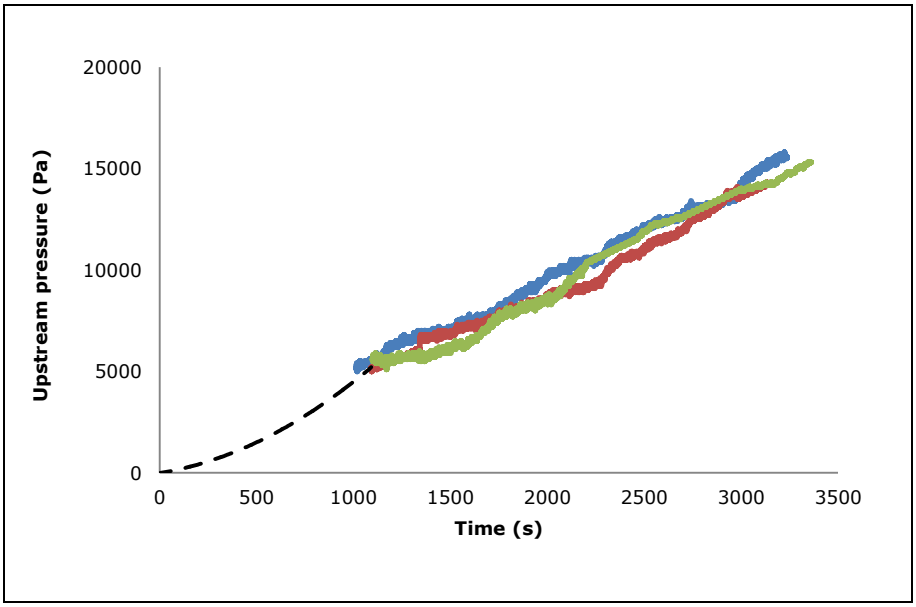


Figure 3.4 Pressure increase in downstream through operation time, ( ) run 1, ( ) run 2, ( ) run 3

In all replicates, a linear tendency was observed after an initial period, indicating the steady state permeation through the sample. In all of the measurements, it took less than 18 min to reach the steady state permeation for the given temperature range, and each measurement could be completed within 1 hour. However, the lag time technique requires several hours because an accurate measurement of the permeation requires evacuation of downstream volume up to high degree of vacuum and requires being confident about the tightness of the system.

The transport coefficients of ethylene oxide, at 48 °C and 3.2 Bar, in surgical drape material are collected in Table 3.1 *(detailed results included in Appendix C).*

Table 3.1 Ethylene oxide transport coefficients, at 48 °C and 3.2 Bar, in surgical drape material

	$\mathscr{P}_{EO}$ (mol s^{-1} m^{-1} Pa^{-1})	D_{EO} (m^2 s^{-1})	S_{EO} (mol m^{-3} Pa^{-1})
Run 1	2.83×10^{-12}	7.63×10^{-9}	3.71×10^{-4}
Run 2	2.63×10^{-12}	8.13×10^{-9}	3.23×10^{-4}
Run 3	2.65×10^{-12}	7.61×10^{-9}	3.48×10^{-4}
Mean ± $CI_{95\%}/2$	$2.70 \times 10^{-12} \pm 2.23 \times 10^{-13}$	$7.79 \times 10^{-9} \pm 5.29 \times 10^{-10}$	$3.47 \times 10^{-4} \pm 4.87 \times 10^{-5}$

CI (Confidence interval)

The lag time experiments allowed the simultaneous determination of permeability, diffusion and solubility coefficients. No publication has been found for EO permeation through membranes, so it is not possible to compare the results obtained with the new apparatus. However, the repeatability of results provided reliability and confidence to the technique.

No correction to permeation data was necessary, because the leak rate of the system was less than 1 % of the flow through the membrane, and it was therefore negligible.

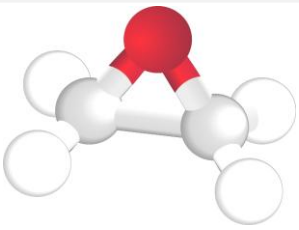
3.4. CONCLUSIONS

A permeation apparatus, which allows experimental determination of permeability, diffusion, and solubility coefficients describing the transport of ethylene oxide through several polymers, has been successfully developed. The design and operation of the apparatus was based upon the principle of measuring a transient change in pressure at conditions of constant volume and temperature and, allows the estimation of the lag time and steady state flow of a gas through membranes.

The performance of the apparatus was assessed and it will contribute to the progress in comprehensive studies about transport properties of EO.

Chapter 4

Understanding the dynamics of ethylene oxide permeation



Understanding the dynamics of EO permeation is required for estimating the sterilisation process effectiveness. However, in loco measurements of EO concentration within a load, during a sterilization cycle, cannot be easily implemented. The work presented in this chapter provides a methodology for validation of the Fickian diffusion process assumed for EO mass transfer through surgical drape loads. Diffusivity was experimentally determined in the apparatus conceived (chapter 3). Ethylene oxide dosimeters were used within the loads of material to be sterilized. Results corroborated the adequacy of Fick´s second law for prediction of EO diffusion through the material. This is a key achievement for determination of the amount of exposure of an article to EO gas.

4.1. INTRODUCTION

EO concentration is one of the requisite process parameters for estimating the lethality of a sterilant cycle. The on-line analysis of EO concentration in the steriliser head-space for parametric release has become a routine practice. However, because of the complexity of controlling EO concentration within the load, where the lethality needs to be estimated, its prediction is a key issue.

The validation of sterilising conditions in all parts of the loads is mandatory and the challenge of EO processes relies in sterilising large industrial loads.

The safety of the process is not under discussion since the densest and the "worst case" load is elected for process validation. However, as EO diffusivity directly interferes with EO concentrations attained within the load, the challenge is to predict and define the times for the qualification cycles and, simultaneously, to predict and assure that the "worst case" load was selected, avoiding unnecessary experimental work that generally precedes the validation studies. This is particularly critical for parametric release since, according to this methodology, the sterilised products are released on the basis of physical measurements, demonstrating that sterilising conditions have been applied. Concern about inadequate sterility at the center of high dense loads may result in the design of cycles that have an excessively long gas dwell time and use large amounts of gas. This situation increases the problem of post-sterilisation residues and prolongs the release time.

The biological indicators are effective in demonstrating whether adequate sterilising conditions are being attained at the load interior. However, they do not provide data on a timely basis nor the means to follow the degree of permeation at different points in the load.

The EO dosimeters can measure the extent of EO permeation at any locations within the steriliser load, since they integrate the EO concentration through exposure time.

The main objective of this work was to correlate the EO concentration in head-space and within the load, using EO dosimeters. This contributes to the validation of the applicability of Fick's second law in EO concentration prediction within loads, which is a key achievement for control and optimisation of the sterilisation of medical devices.

4.1.1. Mathematical considerations

An industrial load normally consists of a large quantity of product encased within several levels of packaging. Typically, the sterile items includes three packaging levels being , (i) individual peel-pack pouch, (ii) shelf-box and (iii) transport box at one level or two levels. Additionally, the product itself might incorporate specific challenges due to intrinsic EO permeation barriers. Each compartment and each packaging layer provides a resistance to permeation that delays the incoming of EO into the product. EO gas in the head-space of the steriliser must penetrate through these successive layers until reaching the product to be sterilised.

The time required for EO permeation through a barrier (*i.e.* packaging layer) is related to the parameter L^2/D where L is the layer thickness and D is the diffusion coefficient (Crank, 1975; Handlos, 1980).

If EO absorption by the packaging layers is not significant, as generally assumed due to the nature of the materials (*i.e.* cardboard, paper and plastic), the permeation time through each layer is expressed by:

$$\text{Permeation time} = \frac{kL^2}{D} \tag{4.1}$$

where *K* corresponds to a factor between 2 to 6, depending on the degree of approach to the initial EO level, *D* is the diffusion coefficient and *L* is the packaging layer thickness (Crank, 1975; Handlos, 1980).

For a three-layer system, and under the same assumption of minimal EO absorption during permeation, the permeation time will correspond to the sum of permeation times for each layer:

$$\text{Total permeation time} = \left[\frac{kL^2}{D}\right]_3 + \left[\frac{kL^2}{D}\right]_2 + \left[\frac{kL^2}{D}\right]_1 \tag{4.2}$$

In practice, for ease of mathematical solution and as the third and the second layer of packaging are soft card boxes (very porous and permeable to ethylene oxide, and therefore irrelevant in terms of permeation barrier), these packaging layers are not considered. As the sterile barrier system ('primary pack') corresponds to a peel-pack pouch constructed of a film (barrier web) and a paper (permeable web), the simple mathematical description of diffusion in a plane sheet of material can be applied. In practice this means that the diffusing substance enter through the plane faces and a negligible amount through the edges. So, EO diffusion occurs in a one-dimensional plane in a medium bounded by two parallel, e.g. the planes at $x=0$, $x=L$. If the region $-L<x<L$ (x corresponding to the position where dosimeter is and L to the thickness) is initially at a uniform concentration C_0, and if the interfaces are kept at a constant concentration C_b, the EO concentration in position x and at time t can be described by the following equation (Crank, 1975):

$$\frac{C - C_b}{C_0 - C_b} = \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{2n+1}\cos\left(\frac{(2n+1)\pi}{2L}x\right)\exp\left(-\frac{(2n+1)^2\pi^2 D}{4L^2}t\right) \quad (4.3)$$

Because the gas concentration in the head-space is uniform and the diffusion on the boundary between the head-space and load is continuous, this concentration is considered to be the same as that on the load surface, C_b.

As gas diffusion proceeds into the load, the EO will be immobilized by the load materials. This process is known as solubility, S of the gas into the material (Çengel, 2006):

$$C_b = S.\mathscr{P} \quad (4.4)$$

where $\mathscr{P}$ is the permeability coefficient.

The ideal-gas law can be applied to EO.

4.2. MATERIALS AND METHODS

4.2.1. Samples and ethylene oxide dosimeters

The EO dosimeters consist of an EO responsive reagent system encased within selectively permeable polymer membranes, which provide a measure of time-averaged EO exposure dose. The material in the capillary column (yellow colour) turns blue when exposure to EO occurs. The EO exposure is continuously recorded and the reading of the colour progression (in a distance scale), done at the end of the sterilisation cycle, provides a visual inspection and a quantitative response of the sterilising gas exposure dose

(Anonymous, 2010). When the colour progression achieves the triangle mark (Figure 4.1), it is indicative that 750 mg L^{-1} h^{-1} of EO exposure occurs (AN1087, Andersen Products, Inc, North Carolina, USA).

The EO dosimeters were placed in the load, into the middle position of peel-packs of surgical drape material (488-103, Bastos Viegas S.A., Portugal; Figures 4.1 and 4.2) and also in the head-space of the steriliser, for confirming EO exposure.

The EO concentration was kept at 255 ± 5 mg L^{-1} and the temperature at 62.5 ± 1.5 °C, above the minimum recommended by the manufacturer of the dosimeter.

Figure 4.1 Photographs of the surgical drape material and EO dosimeter

Figure 4.2 Photographs of surgical drape material in its shelf-box

4.2.2. Sterilisation process

The experiments were performed in a standard EO chamber (21059C Sterichem, France) of approximately 3 m^3 with controlled temperature, EO concentration and humidity. The cycle design corresponds to a standard cycle, similar to the ones described in chapter 2. The conditions were kept constant through different exposure times and were monitored by adequate equipment fitted to the chamber (process variables included in Table*D.1.1* and *D.1.2* of *Appendix D*). The EO concentration was assessed by an infrared analyser in the steriliser chamber and corresponds to a condition obtained in the steriliser head-space.

4.3. RESULTS AND DISCUSSION

The AN1087 dosimeter was designed for controlling EO sterilisation cycles since it integrates variables that influence the sterilisation: EO concentration, length of exposure to the gas and the temperature on the sterilisation load. In this study, the temperature of the cycle was kept constant and above the recommended one, which allowed to correlate the colour progress in EO dosimeters (Figure 4.3) directly with EO concentration and length of exposure to the gas.

The correlation between the dosimeter response and EO concentration of exposure indicated by the manufacturer is in agreement with the results achieved with the dosimeters placed in the steriliser head-space, where the infrared analyser is connected (Figure 4.3). As obvious, the same direct response is not applicable within the load and, the equation that relates the colour progress in EO dosimeters, *y*, with EO concentration,

x, is y = 4.41x10^{-3}x + 1.34. The cycle parameters and results are included in Table *D.1.1* of *Appendix D*.

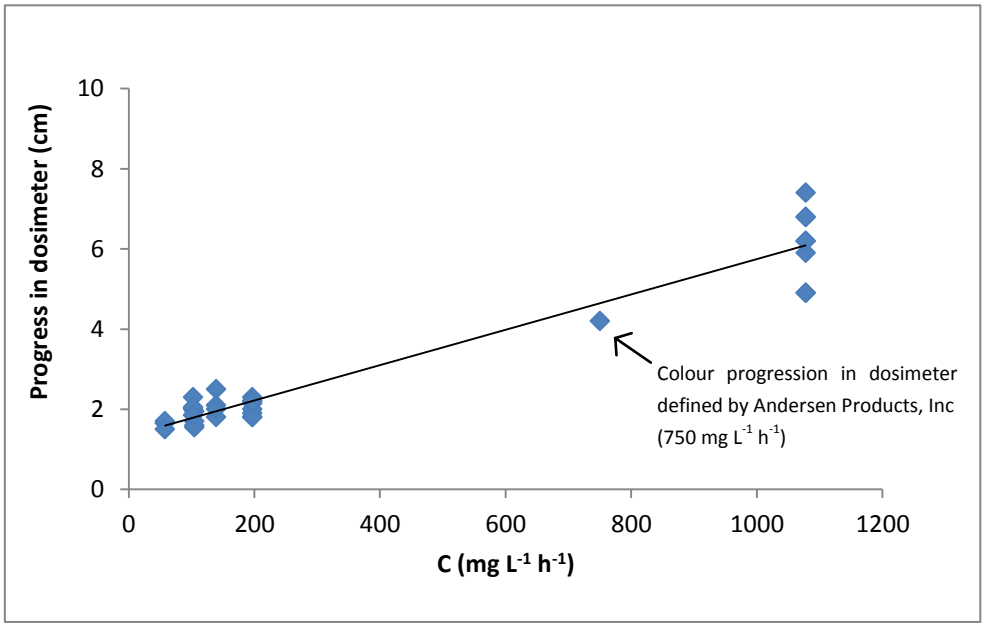


Figure 4.3 Colour progress in EO dosimeters placed in the steriliser head-space *versus* EO concentration

Table *D.1.2* of *Appendix D* summarizes the colour progress in EO dosimeters within the load for the corresponding sterilisation cycles. Using the previous linear expression that relates the EO dose with the colour progression in the dosimeter, the estimated concentrations within the load were obtained (Table *D.2* of *Appendix D*).

In parallel, the estimation of EO concentration by Fick's second law of diffusion (eq. 4.3) was carried out, since the diffusivity is a known parameter (7.79 x 10^{-9} m^2 s^{-1}, *Chapter 3*). Those predicted values of EO concentration (Table *D.3.1* of *Appendix D*) were in agreement with the ones obtained by dosimeters integration (Figure 4.4; R^2=0.92). This achievement demonstrates that the model was appropriate for describing the kinetics of the sterilisation permeation.

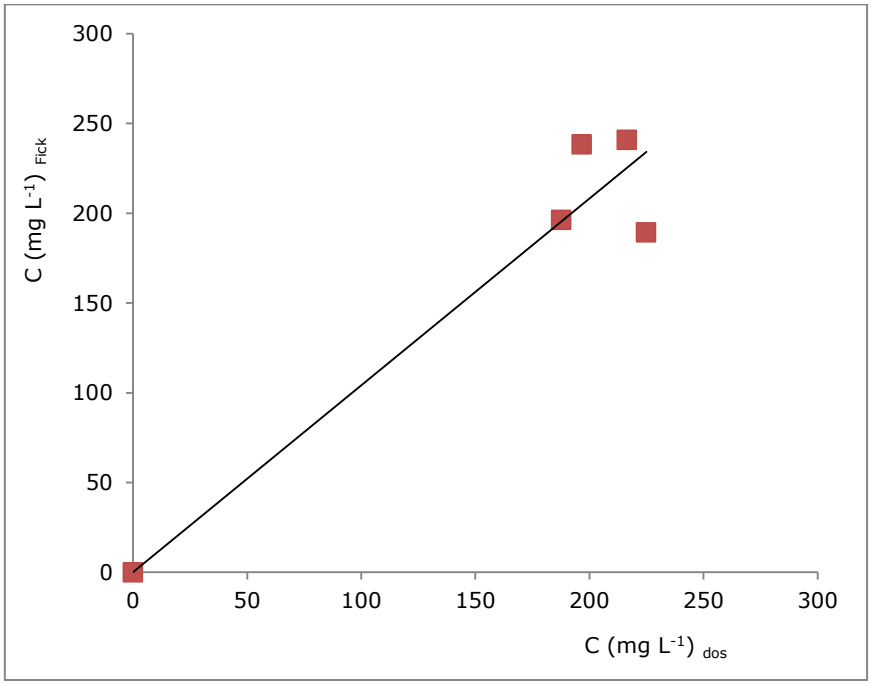


Figure 4.4 Concentration of EO (within the load) predicted by Fick's second law of diffusion *versus* concentration of EO achieved by dosimeters integration

The results indicate that the diffusion of EO in the surgical drape material is Fickian and from the knowledge of the transport coefficients, one can calculate the EO concentration within the load to sterilise, throughout the sterilisation exposure time.

Variations in load elements, such as in packaging and raw materials, may alter the process behaviour.

Using the Fickian model, and taking in account the diffusion coefficient temperature dependence, EO concentration was estimated for the five runs of chapter 2 with target temperatures around 60 °C. Figure 4.5 displays the progress in terms of EO concentration within load material through exposure time, simultaneously with the inactivation curves of *B. subtilis* spores (included in chapter 2). The cycle parameters and the final EO concentration within the load achieved by Fick's second law of diffusion are included in Table *D.3.2* of *Appendix D*.

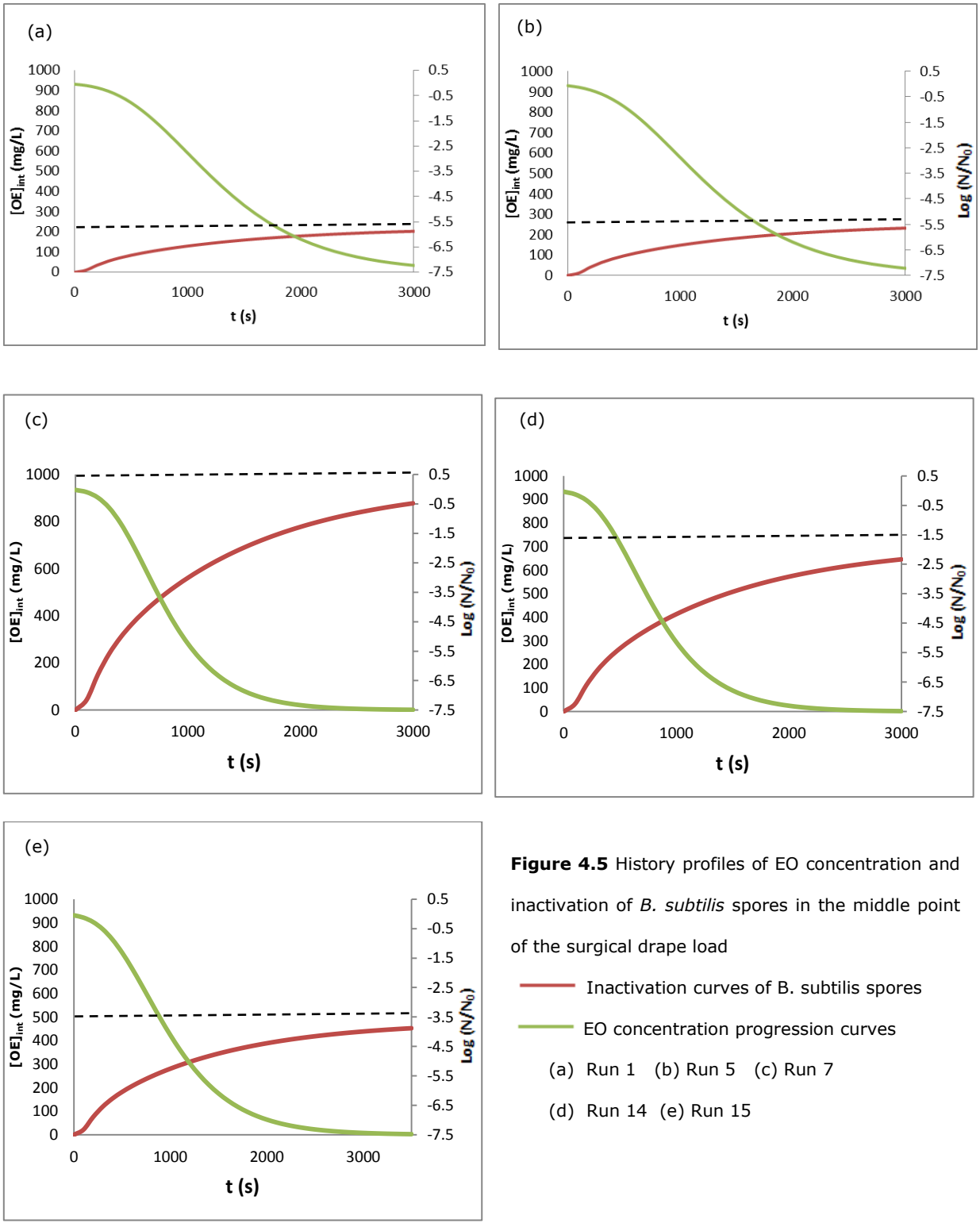


Figure 4.5 History profiles of EO concentration and inactivation of *B. subtilis* spores in the middle point of the surgical drape load

Inactivation curves of B. subtilis spores

EO concentration progression curves

(a) Run 1 (b) Run 5 (c) Run 7

(d) Run 14 (e) Run 15

The concentration within load material gradually increases, approaching the EO concentration head-space level, shown as dotted lines in Figure 4.5.

No evident correlation was found between the histories of EO concentration inside the materials and the lethality curves.

4.4. CONCLUSION

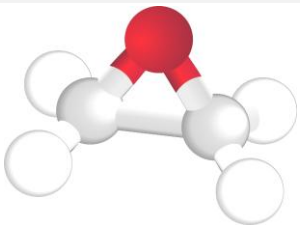
The kinetics of gas permeation through EO sterilisation was successfully predicted by Fick's second law of diffusion, using diffusion coefficients obtained in the apparatus developed. This is a key step for determining the EO concentration exposure of an article.

The combination of product and packaging material, as well as the configuration and density of the load, determines the permeation rate. The design and validation studies must disclose this topic, thereby allowing the optimisation of packaging layers and steriliser configurations to achieve maximum sterilising efficacy.

The approach herein presented enhances the science of EO sterilisation. The methodology allows optimising the EO cycle design to achieve sterility and lowest residues, shortening the release time. It may also help reduce the total time required to validate a particular sterilisation cycle.

Chapter 5

Kinetics of ethylene oxide desorption from sterilised materials



Medical devices sterilised by ethylene oxide must be properly aerated to remove residual gas and by-products. In this chapter, kinetics of ethylene oxide desorption from different sterilised materials were studied in a range of aeration temperatures. The experimental data was well described by a Fickian diffusion mass transfer behaviour, and diffusivities were estimated for two textile and two polymeric materials, within the temperature range of 1.5 to 59.0 °C.

The results will allow predictions of ethylene oxide desorption, which is a key step for the design of sterilisation/aeration processes, contributing to an efficient removal of residual ethylene oxide content.

5.1. INTRODUCTION

One of main disadvantages of the ethylene oxide sterilisation methodology is its potential hazard to patients due to exposure to devices that have been improperly aerated. The toxicological concern of ethylene oxide to patient health (and eventually of its degradation products) makes its prediction and control an essential matter (Tock and Chen, 1974; Romano and Renner, 1975; Handlos, 1980; Muzeni, 1985; Gibsonet al., 1989; Danielson et al., 1990).

The EO sterilisation process includes an aeration step that should reduce the residuals to a safe level. Different aeration technologies have been reported, such as pulsed vacuums post process and heat addition, steam addition and removal, as well as combinations of different gases and pressure set points, and newer developments such as microwave desorption. Despite the major interest on this issue, there are no recent published studies about efficiency comparisons for the different aeration technologies, and thermal aeration remains the methodology of choice (Matthews et al., 1989; Peacock, 1999; Sordellini et al., 2001; Strain and Young, 2004; Strain et al., 2004).

The goal is to keep compliance with the maximum limits defined by ISO 10993-7 (the new version increased the stringent requirements), which is a current challenge for EO sterilisation facilities. This standard is included in a series governing the biological testing of medical devices. It specifies the maximum allowable limits of EO and ethylene chlorohydrin, based on toxicological risk of the residue to patient, according to the length of the time the patient is likely to be exposed to the device (limited exposure - daily, prolonged exposure - monthly, permanent exposure, and special situations) (AAMI TIR 19, 1998; AAMI TIR 19/A1, 1999; ISO 10993-7, 2008).

The product release to the market can be done by measuring the residuals and verifying the compliance of the product with the maximum allowable residue, or by using dissipation curves (*i.e.* concentration of EO in the product *versus* aeration time). According to the dissipation curves methodology, the product release is based on the required aeration time for attaining the maximum allowable EO residue (ISO 10993-7, 2008).

The EO desorption is mainly dependent on the product (including composition, configuration and also packaging material), and on the sterilisation and aeration cycle design.

The aeration of medical devices is a diffusion-controlled process. The theoretical background for using the diffusion theory is well-established and, with a convenient experimental design, it is possible to predict the EO desorption rate for different materials (White and Bradley, 1973; Tock and Chen, 1974; Handlos, 1980; Gibsonet al., 1989).

Current studies show that the mechanical aeration at higher temperatures allows easier desorption of EO residues, but the point is how to predict this behaviour. The goal is to achieve the minimum EO residuals whilst guaranteeing sterilisation.

The purpose of the present work was to estimate ethylene oxide diffusion coefficients for aeration processes at different temperatures. Different materials used in the medical field (textile and polymers) were tested.

5.1.1. Mathematical considerations

The EO desorption from materials can be assumed as a simplification of a complex diffusion process. Mass transfer from one point of the system to another is the result of

molecular motions induced by concentration gradients. In view of the one-dimensional diffusion model, the diffusion takes place on both directions through the plane sheet of material (faces which may be considered normal to the x-axis, and a negligible amount through the edges). If the diffusion coefficient is assumed to be constant, the process may be described by Fick's second law (Crank, 1975):

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \tag{5.1}$$

where *C* is ethylene oxide concentration of the material at position *x* and after a time *t*; and *D* is the diffusion coefficient.

If the process is controlled by internal diffusion and the solute concentration at the material surface remains constant with time, the amount of EO lost into the surrounding medium after a time *t* can be calculated from:

$$\frac{\overline{C}}{\overline{C_0}} = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-\left\{\frac{(2n+1)\pi}{2L}\right\}^2 Dt\right] \tag{5.2}$$

where $\overline{C}$ is the (average gas) concentration in the material (the index 0 is related to the initial value), and *L* is half of the thickness of the material (White and Bradley, 1973; Tock and Chen, 1974; Crank, 1975; Handlos, 1980; Vink and Pleijsier, 1986; Phatak et al., 1987; Gibsonet al., 1989).

The diffusion coefficient *D* is often correlated with temperature (*T*) by an Arrhenius-type behaviour (Handlos, 1980):

$$D = D_R e^{-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_R}\right)} \tag{5.3}$$

herein D_R is the diffusivity at a finite reference temperature T_R, E_a the activation energy and R the universal gas constant.

5.2. MATERIALS AND METHODS

5.2.1. Experimental procedures

Materials

Two different polymers (i.e. polypropylene, L 9.56×10^{-4} m – galipot, 489-312, Bastos Viegas S.A., Portugal; and polyvinylchloride, L 2.82×10^{-3} m – nasal aspirator pear, 127-007, Bastos Viegas S.A., Portugal) and two different textile materials (i.e. viscose/polyester, L 4.50×10^{-4} m – baby blanket, 454-300, Bastos Viegas S.A., Portugal; and viscose/polyethylene, L 1.84×10^{-4} m – surgical drape, 488-103, Bastos Viegas S.A., Portugal) were tested. Materials were cut into small plane sheet pieces with about 1 g and weighed. After, samples were placed in paper sterilisation bags (448-301, Bastos Viegas S.A., Portugal) and sterilised by EO according to the conditions described below.

Sterilisation and aeration processes

The samples were sterilised in a standard EO chamber (21059C Sterichem, France) for approximately 3 hours. Ethylene oxide concentration, temperature and relative humidity conditions were 450 mg L^{-1}, 50 °C and 70 %, respectively. After sterilisation, samples

were removed from the EO chamber and placed into three different aeration cabinets simulating low, medium and high temperature aeration processes (GDM-72, True Manufacturing Company, USA; B-5050, Heraeus Oven, Germany; and 1600 HAFO SERIES 1685, VWR, USA, respectively) with controlled temperature and uniform air flow rate. The temperatures of the aeration cabinets were 1.8 ± 0.3, 30.7 ± 1.9 and 58.9 ± 0.1 °C and replicate samples were taken at different time intervals for extraction and analysis. Determination of EO residues in the products was carried out according to the analytical procedure below described.

Ethylene oxide determination

The ethylene oxide content in the samples was assessed by exhaustive extraction and subsequent analysis via gas chromatography by head-space methodology, as described in ISO 10093-7 (Romano and Renner, 1975; De Rudderet al., 1984; Kaye and Nevell, 1985; Marlowe et al., 1987; Sordellini et al., 2001; Strain and Young, 2004), and as follows:

Exhaustive extraction and gas chromatographic analysis

The samples were sealed in vials of 20 mL (20-2000, Microliter Analytical Supplies, USA) using crimp seal with polytetrafluoroethylene septum cap closures (20-0051, Microliter Analytical Supplies, USA). They suffered a 60 minutes thermal extraction at 100 °C, allowing the volatile compounds to go into the vial head-space. A heated gas-tight syringe (1001 CTC $_{(23/5)}$, 203082, Hamilton, Swiss) was used to remove a 100 µL head-space aliquot, which was directly injected into the gas chromatograph column.

Instrumentation

A gas chromatograph (CP-3800, VARIAN, USA) equipped with a flame-ionization detector (FID 3800, VARIAN, USA) and a head-space auto sampler (PAL System, Combi PAL, USA) was used. The instrument operated isothermally: the column (CP-WAX 52CB, VARIAN, USA) at 40 °C, the injector at 200 °C and the detector at 250 °C. Helium was used as the carrier gas with a flow rate of 1 mL min^{-1}, while a flow rate of 300 mL min^{-1} was used for air, 30 mL min^{-1} for hydrogen and 35 mL min^{-1} for nitrogen, as makeup.

The injection of the 100 µL aliquot was done in the split mode (1:10 split ratio).

Quantification was achieved by electronic integration of the output peaks areas from the gas chromatographic detector, having the same retention time as EO standard.

Preparation of the ethylene oxide standard

The EO standard (03901 lot 1395053, Fluka, USA) and its dilution were performed according to a reference test method defined by ISO 10993-7 for the head-space methods. From the standard ethylene oxide solutions injected and calculated peak areas, a calibration curve was plotted.

5.2.2. Modelling procedures

Diffusivity was estimated by non-linear least squares regression analysis, fitting the Fickian model (eq. 5.2) to experimental data. A program was written in FORTRAN 77

language (Fortran 5.1, 1990) and the Simplex method was used for minimization of the sum of the squares of the residuals (Nelder and Mead, 1965).

Diffusion parameters (D_R and E_a; eq. 5.3) were also estimated by non-linear regression analysis (SPSSInc. 15.0, 2006). The reference temperature assumed was the average value of the range considered per material.

The quality of the regressions was assessed by residuals analysis (normality and randomness) and by the coefficient of determination R^2. The precision of the diffusion parameters estimates was evaluated by confident intervals at 95 %.

5.3. RESULTS

Table 5.1 presents the initial EO samples concentration. The desorption curves of ethylene oxide for the materials under study are included in Figure 5.1 (plots of ethylene oxide concentration ($\bar{C}$), normalized in relation to initial values ($\overline{C_0}$), versus aeration time, normalized in relation to square thickness (L^2), seeking diffusional process comparison for different materials at the aeration temperatures imposed). The results obtained are included in *Table E.1.1 to E.1.11 of Appendix E*. The Fick's-derived model (eq. 5.2) was used to fit to the experimental data and diffusivities were estimated for the materials and conditions tested (values included in Table 5.2). The results of the dependence of diffusivity on temperature are represented in Figure 5.2, and the diffusivity parameters (D_R and E_a) estimated assuming the Arrhenius model (eq. 5.3) are also in Table 5.2. Precision of the parameters was assessed by calculating the confidence intervals of the parameters at 95 %.

Table 5.1 Initial ethylene oxide concentration (C_0) and corresponding half of the thickness (L) of the sample material

Material	T (°C)	C_0 ($mg_{EO}\ g_{device}^{-1}$)	L (m)
polypropylene	58.8 (high)	3.08×10^{-1}	
(galipot, 489-312)	32.6 (medium)	1.01×10^{0}	9.56×10^{-4}
	1.9 (low)	1.01×10^{0}	
polyvinylchloride	59.0 (high)	2.12×10^{0}	
(Nasal aspirator pear,	32.2 (medium)	9.30×10^{-1}	2.82×10^{-3}
127-007)	1.7 (low)	1.13×10^{0}	
viscose/polyethylene	32.0 (medium)	4.81×10^{-3}	1.84×10^{-4}
(surg. drape, 488-103)	2.0 (low)	4.73×10^{-3}	
viscose/polyester	28.8 (medium)	2.76×10^{-1}	
(baby blanket, 454-300)	22.2	2.74×10^{-1}	4.50×10^{-4}
	1.5 (low)	2.76×10^{-1}	

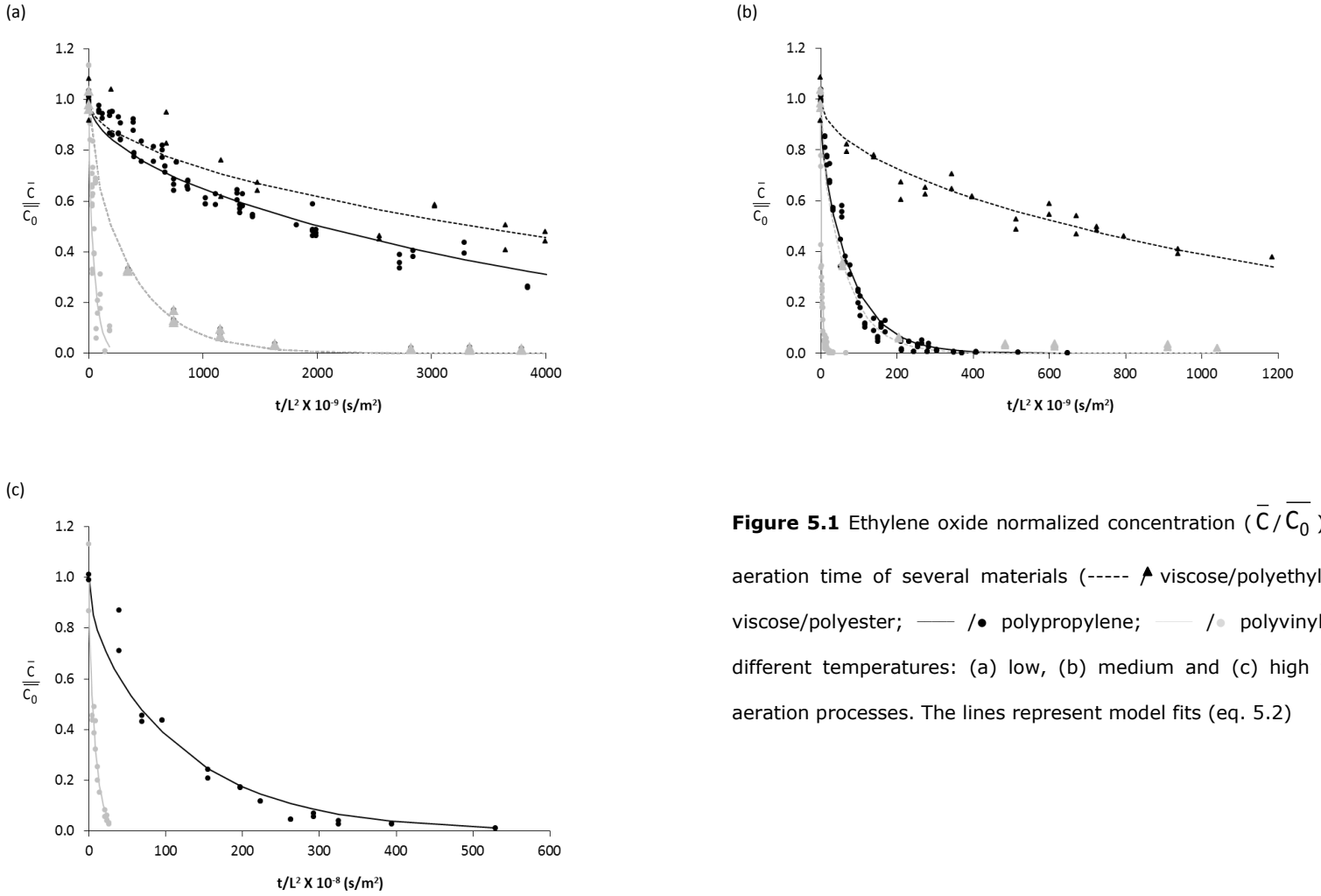


Figure 5.1 Ethylene oxide normalized concentration ($\bar{C}/\bar{C_0}$) throughout aeration time of several materials (----- /▲ viscose/polyethylene; ----- /▲ viscose/polyester; —— /● polypropylene; —— /● polyvinylchloride) at different temperatures: (a) low, (b) medium and (c) high temperature aeration processes. The lines represent model fits (eq. 5.2)

Table 5.2 Ethylene oxide diffusion coefficients for the materials tested

Material	T (°C)	D ($m^2 s^{-1}$)	T_R (K)	D_R ($m^2 s^{-1}$)	±	$CI/2_{95\%}$	E_a (kJ/mol)	±	$CI/2_{95\%}$	R^2
polypropylene	58.8 (high)	$7.769x10^{-12}$								
(galipot, 489-312)	32.6 (medium)	$1.225x10^{-12}$	304.25	$1.081x10^{-12}$	±	$7.641x10^{-13}$	59.8	±	21.69	1.000
	1.9 (low)	$2.429x10^{-14}$								
polyvinylchloride	59.0 (high)	$1.118x10^{-10}$								
(Nasal aspirator pear,	32.2 (medium)	$2.700x10^{-11}$	304.12	$2.500x10^{-11}$	±	$2.203x10^{-11}$	45.41	±	27.59	0.999
127-007)	1.7 (low)	$1.857x10^{-12}$								
viscose/polyethylene	32.0 (medium)	$7.444x10^{-14}$	290.15	$3.436x10^{-14}$	±	*	37.94	±	*	*
(surg. drape, 488-103)	2.0 (low)	$1.457x10^{-14}$								
viscose/polyester	28.8 (medium)	$1.451x10^{-12}$								
(baby blanket, 454-300)	22.2	$8.916x10^{-13}$	290.65	$6.737x10^{-13}$	±	$6.272x10^{-13}$	48.69	±	67.98	0.996
	1.5 (low)	$2.451x10^{-13}$								

* Meaningless value

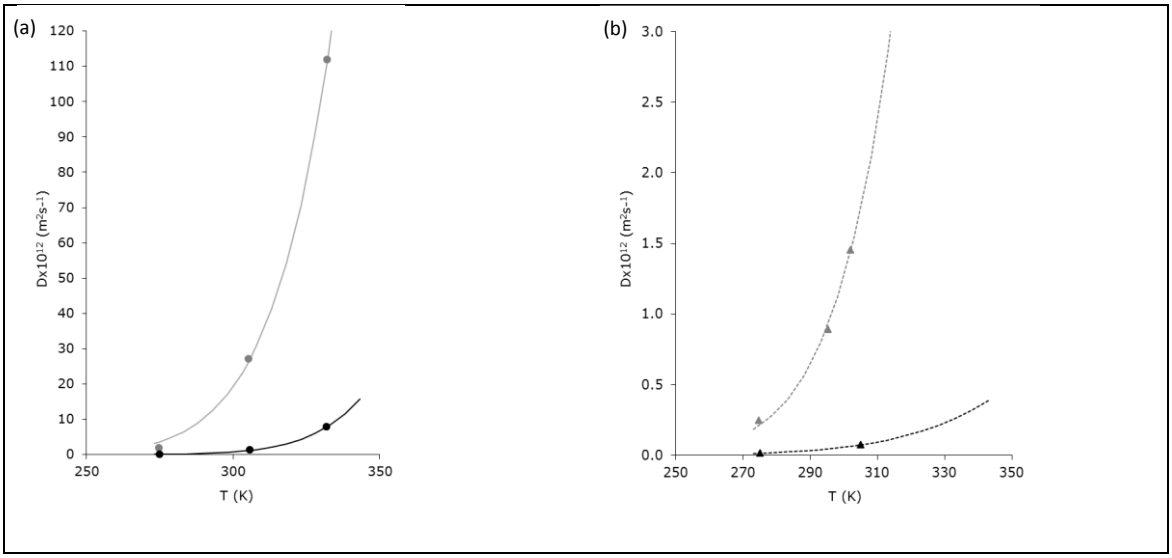


Figure 5.2 Dependence of diffusivity on temperature for (a) polymer (● polyvinylchloride; ● polypropylene) and (b) textile materials (▲ viscose/polyester;▲ viscose/polyethylene). The lines represent model fits (eq. 5.3)

5.4. DISCUSSION

The results showed that the ethylene oxide desorption from different materials follows a Fickian diffusion kinetics. There is a preliminary stage where a faster EO rate of desorption is observed, after which the rate of desorption is slower tending to an asymptotic value (Figure 5.1). This is more evident for the higher aeration temperatures studied (that include typical environmental and aeration operating temperatures). Ethylene oxide diffusion coefficients of the tested materials were estimated by fitting the Fickian model to experimental data (i.e. $\overline{C}/\overline{C_0}$ - versus aeration time; eq. 5.2). The adequacy of the model was assessed by residuals analysis (normality and randomness

were verified) and by the coefficient of determination R^2,which were above 0.99 in all cases.

The estimated diffusivities of the polymeric samples were of the same magnitude of the ones reported in the literature for similar materials (White and Bradley, 1973; Handlos, 1980). Regarding the textile materials, and due to lack of published data, comparison of the results is limited.

The majority of studies related to EO sterilisation dismissed the mass transfer phenomenon that rules the process, and assumes a simple first order kinetics (i.e. the logarithmic variation of EO concentration, *lnC* with aeration time is assumed to be linear) (ISO 10993-7, 2008). However, and as verified in the materials/aeration conditions studied in this work, such linear behaviour does not describe experimental data tendency (especially for the initial phase of the aeration process).

Previous studies demonstrated that the differences of EO diffusivity estimated for different materials is due to their surface area throughout mass transfer occurs, which is related with its physical structure. Generally speaking, materials with increasing crystallinity and density will show lower diffusion coefficients (Handlos, 1980; Scott, 1982; Aeschlimann, 1984; Muzeni, 1985; Vink and Pleijsier, 1986; Bubenet al., 1999; Lucaset al., 2003). The dependence of diffusivity on aeration temperature is presented in Figure 5.2. Such dependence was assumed to be described by the Arrhenius eq. 5.3 and diffusion coefficients, D_R and E_a, can be compared. The adequacy of Arrhenius model was verified by residual analysis and coefficient of determination (above 0.99 in all cases). The reduced number of experimental points did not compromise precision of the estimated diffusion parameters. The viscose/polyethylene material (surgical drape) was the only material tested at two aeration temperatures and this was due to its intrinsic low concentration of EO residues in time zero (close to the method detection limit) and also to its fast dissipation. With exception of viscose /polyester material (baby blanket), with an E_a

standard error at 95 % higher than the estimate itself, for the remaining materials diffusion parameters were estimated with satisfied precision.

The diffusion coefficients estimated in this work allow prediction of minimum aeration time required to reduce EO content of a specific material (with different geometries) to a desired level. Such information will contribute to a more efficient process design and further, the selection of materials to be sterilised.

5.5. CONCLUSIONS

Ethylene oxide diffusivities through different materials were successfully estimated. This is certainly a valuable tool for predicting EO dissipation at standard temperatures during the aeration process. This is a key step on the overall optimisation of EO sterilisation, since the wise choice of the parameters would result in a fast elimination of EO residuals from the material.

The results were obtained for unpacked and individually aerated devices and will be profitable in predictions of packed and staked sterilised devices throughout distribution and storage.

Chapter 6

General Conclusions and Suggestions for Further Work

This chapter summarises the main conclusions and major outcomes of the work reported in this dissertation. The strategies for optimisation of the ethylene oxide sterilisation of medical devices are highlighted and directions for further work are outlined.

6.1. GENERAL CONCLUSIONS

The main goal of the present thesis was to provide a better understanding of the EO medical devices (sterilization) process, by development of mathematical models for prediction of EO diffusion and microbial lethality. This was achieved and therefore the dissertation provides (i) a mathematical model for the assessment of influencing variables on the microbial inactivation, (ii) an accurate prediction of sterilising EO concentration within the load (by study of EO diffusion) and (iii) the assessment of the temperature effects on outgassing kinetics.

Temperature, ethylene oxide concentration and humidity are described as the most relevant variables on microbial inactivation kinetics using EO. An extensive study using a wide range of operational conditions was developed and the conditions were simulated throughout different sterilisation times in a sterilisation chamber, with a load of surgical drapes. Using *B. subtilis* as target microorganism, it was observed its inactivation varied from a linear tendency (first order kinetics) till a pronounced sigmoidal shape, depending on the studied variables. As inactivation rate increases, the shoulder decreases and the Gompertz model was successfully applied in data fitting. Results showed that within the limits of 45-90 %, the RH did not influence significantly the lethality and, although the temperature was the main factor influencing the kinetic parameters, also the EO concentration significantly influenced the *B. subtilis* inactivation. The effect of these two variables on kinetic parameters (shoulder and maximum inactivation rate) was accurately described by a Gompertz model. Overall, an inactivation model expressed only in terms of the relevant process variables was achieved.

The current study provides a method of integrating lethality and its adequacy in predicting *B. subtilis* inactivation was demonstrated. The obtained results highlight the importance of adequate equipment for controlling all the parameters of the process, as

well as the definition of appropriate process variables, thus contributing to its design optimisation.

The conventional design of EO sterilisation cycles usually involves a significant amount of experimental work, which is time consuming and costly. This work is certainly a contribution for an efficient control, design and optimisation of the EO sterilisation process.

In parallel, and as the EO concentration is only monitored in the steriliser head-space, although the sterilisation effectiveness is related to the EO concentration attained within the load, its prediction is a key issue for theoretical estimation of process lethality and devices EO residuals.

An apparatus was designed and conceived for experimental determination of EO diffusivity, permeability and solubility coefficients through different materials of medical devices. The design and operation of the developed apparatus was based upon the principle of measuring a transient change in pressure at conditions of constant volume and temperature (variable-pressure approach) and, allowed the estimation of the lag time and steady state flow of a gas through membranes. Supported on Fick's laws of diffusion the transport properties of EO were determined for surgical drape material from three repeatable experiments.

Based on such transport coefficients, modelling of EO permeation through an industrial load of the same material was attained. Fick’s second law of diffusion was applied to estimate EO concentration within the load. This is a key issue for estimation of process lethality and EO residuals in the devices.

The model proved to be accurate in evaluating the kinetics of EO gas penetration through the load (and prediction of EO concentration within the products). The results were validated by its agreement with the results attained with EO dosimeters, which integrate

EO concentration through exposure time. The developed approach proved to be effective in determining the amount of exposure of an article to the EO gas.

Additionally, due to the fact of being a chemical sterilisation process, the dichotomy sterilisation and aeration needs to be set out from the outset. The overall optimisation of EO sterilisation relies on achieving sterility and the lowest residues. Due to toxicological concern of EO to patient health, sterilising with low EO concentrations and improving aeration is an essential matter. The goal is to keep compliance with the maximum allowable EO and ECH limits defined by ISO 10993-7 whilst guaranteeing sterilisation.

The aeration of medical devices is a diffusion-controlled process. The study carried out allowed obtaining EO diffusion coefficients for aeration processes at different temperatures, and it is certainly a valuable tool for predicting EO desorption at standard temperatures of the aeration process. The experimental data is consistent with a Fickian diffusion transport process and diffusivities were estimated for materials used in the medical field (textile and polymers), within the range of 1.5 to 59.0 °C.

The complete approach herein presented enhances the science of EO sterilisation.

The research gathered in this dissertation is a step further to contribute to the design of an effective and efficient controlled EO sterilisation of medical devices, while maintaining its functionality and safety.

With this work, the parametric release, that is the assessment of sterilisation adequacy based on physical parameters measurement, was also much more scientifically supported.

6.2. SUGGESTIONS FOR FURTHER WORK

From the research findings of this dissertation, the following topics for future research can be identified:

(i) Development of reliable risk assessment analysis for EO and related residuals;

(ii) Experimental determination of EO diffusivity through different materials of medical devices, using the apparatus developed and conceived in Chapter 3;

(iii) Extend the modelling of diffusion to other boundary conditions and to other media also with different geometry; the robustness of these designs should be tested;

(iv) Further developments about the efficacy and equivalency among different aeration methodologies;

(v) Definition of a global and integrated modelling approach that merges mass transfer phenomena and inactivation kinetics with the purpose of obtaining an accurate estimation of microorganisms' lethality. Based on the conditions imposed in the sterilisation chamber, the conditions occurring within the load could be predicted;

(vi) Study of microbial inactivation kinetics, by using EO as sterilising agent, in packs of MDs (combinations of medical devices, materials and packaging that are placed into a single complex pack).

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